EXPERIMENTAL STUDIES ON MYOCARDIAL BLOOD FLOW AND METABOLISM WITH SPECIAL REFERENCE TO HYPERBARIC OXYGEN

by

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Introduction

The use of air at high pressure has a long history in the annals of medicine. After an initial flush of enthusiasm, interest in this topic waned although some physiological investigations on the effects of high ambient pressures were necessary for military purposes both in submarine warfare and also in deep sea diving. When oxygen became commercially available as a therapeutic instrument in the 1920's interest was focused on the use of oxygen at higher pressures than normal in the treatment of various disorders. A good review (Jacobson, et.al. 1964) is available of the history of the use of hyperbaric oxygen and the endeavours to manipulate it in the treatment of various organs and systems. In the last twenty years efforts have been made to use this mechanism in the treatment of various forms of heart disease.

Early experimental work on animals seemed to demonstrate that there was possible benefit in the experimental infarct situation with special reference to diminution of liability to arrhythmias (Smith and Lawson, 1962). Work was also put forward which suggested that perhaps the area of infarcted tissue might be limited (Trapp and Creighton, 1964). Studies on human myocardial infarction were

undertaken and have been reported (Cameron, et.al.,1965; Kenmure, et.al., 1968). However, it became increasingly clear that an understanding of the fundamental changes in myocardial blood flow and metabolism which occurred on exposure to high pressures of oxygen was lacking and little well documented experimental work was available. The work which had been done initially had used experimental preparations which in the main necessitated a thoracotomy in the experimental animal and although this provided valuable information, a method which could be used in closed chest animals would obviously be preferable.

It was the object of the investigation which is here reported to assess methods of measuring myocardial blood flow and to attempt to find a method which would be suitable for work in a hyperbaric environment. The pressure chamber at the Western Infirmary and the experimental laboratories of the Department of Surgery, the Western Infirmary, Glasgow, in which this work was to be done, were of sufficient scope to enable sophisticated investigations to be undertaken. (Figure 1).

The method finally selected involved the estimation of myocardial blood flow by the direct injection of a radio-active gas ^{133}Xenon into the coronary arteries. When this method was well established and tested a programme of investigation was then drawn up. This included measurement of the effects of oxygen at high pressures on myocardial blood flow and metabolism. When the changes

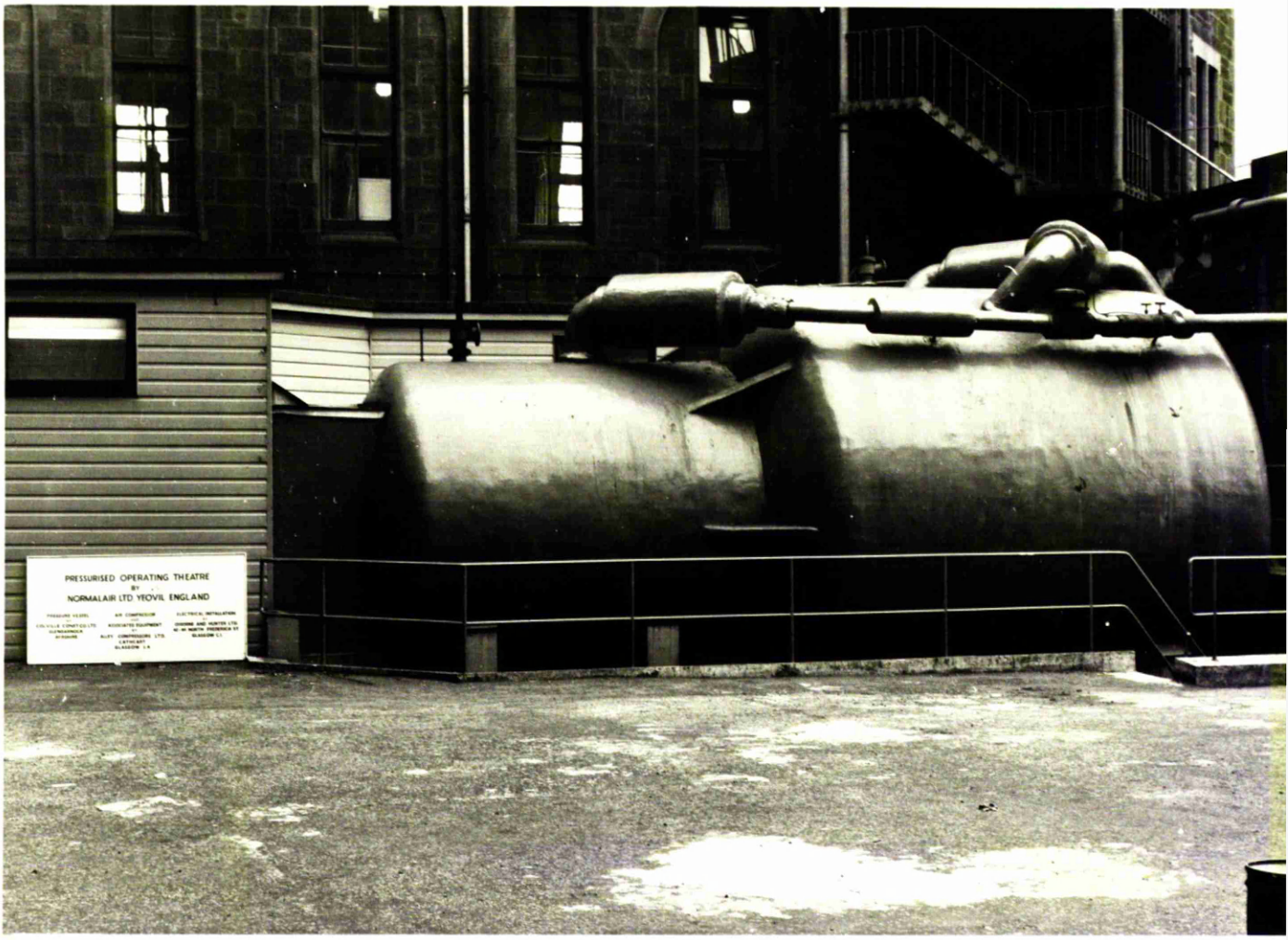

Figure 1: External view of the pressure chamber at the Western Infirmary, Glasgow.

observed were established beyond doubt, a combined physiological and pharmacological investigation was undertaken to determine the methods by which these changes were brought about. Work was also done on the changes in myocardial blood flow with exposure to carbon dioxide and later investigation was made on the effect of carbon dioxide combined with oxygen at high pressures. Eventually, as a result of the early experiments, an investigation was made into the effects of oxygen prolonged to the length of time which was currently being used in clinical practice and the results of these investigations are presented.

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Chapter 1

THE HISTORY OF INVESTIGATIONS OF MYOCARDIAL BLOOD FLOW

Until very recently, practically all the knowledge concerning myocardial blood flow has been gained by different applications of the principle enunciated by Adolf Fick in 1870. The title of his communication was "Über die Messung des Blutquantums in den Herzventrikeln", and this was published in the Proceedings of the Wurzburg Physikalische-Medizinische Gesellschaft for July 9th, 1870.

This highly important communication, possibly one of the most important ever in physiology, reported his method for measuring the amount of blood ejected by the ventricle of the heart with each systole. The basic principle was that the cardiac output could be calculated from the total oxygen absorbed per minute divided by the uptake of oxygen into the blood per unit of blood flowing, i.e. the arterio-venous oxygen difference. This simple statement of relationships was important since for the first time it brought together notions of blood flow and respiratory gas transport. Equally important was the fact that it gave the essential expression of the dilution principle of blood flow measurement, i.e. the faster the blood flow, the less oxygen taken up per unit of blood flowing. This dilution principle is the basis of most of the accepted methods of measuring not only cardiac output but also the flow of blood to organs and is the underlying principle of the

technique of measurement of myocardial blood flow used in this present work, i.e. the myocardial clearance of radio-active Xenon.

Seneca, the Roman philosopher, had no knowledge of matters such as these when he gave an excellent description of the clinical picture of angina pectoris and the link between the structure and function of the coronary arteries had to await many centuries. The coronary arteries were drawn by Leonardo de Vinci and also illustrated in the Fabrica of Vesalius although at that stage it was still believed in the Galenical tradition that the heart was nourished by the coronary veins.

William Harvey had realised the function of the coronary vessels as he noted in De motu cordis (1628). "Besides if the blood could permeate the substance of the septum or could be imbibed from the ventricles what use would there be for the coronary artery and vein, branches of which proceed to the septum itself to supply it with nourishments." At the end of the seventeenth century the coronary circulation had been described and its functions recognised. Pierre Chirac (1698) noted that ligation of the coronary arteries caused cardiac arrest and in 1761 the pathology of the coronary arteries was delineated by Morgagni. In the eighteenth century clinical contributions were made by John Hunter, Jenner, Parry and Heberden but special mention must be made of Allan Burns whose book "Observations on some of the most frequent and important diseases

of the heart" (1809) contained an excellent physiological account of the syndrome of coronary insufficiency. Direct experimental work on this topic awaited the investigations of Marshall Hall (1842) and John Erichsen (1842). Erichsen ligated the coronary arteries of dogs and measured the mean duration of subsequent ventricular action.

In Germany, Von Bezold and Greyman in 1867 charted the effect of coronary ligation of rabbits hearts and Panum (1862) had injected all the coronary arteries with opaque material and then observed cardiac function. Julius Cohnheim in 1881 performed animal experiments with coronary artery ligation and used manometers to record arterial pressures during the experiment.

Studies on the relationship between flow in the coronary arteries and cardiac activity had been investigated physiologically since the mid nineteenth century. Rebatel in 1872 described his findings in horses and mules while Newell-Martin and Hedgvick in 1882 used graphical means to record the blood pressure in carotid and left coronary artery in the dog. The latter found that "whether arterial pressure be high or low every feature of the carotid pulse occurs simultaneously in the coronary". Further advances in knowledge depended on the refinements of technique for measuring pressures and development of new methods of measuring flow. Cohn and Romiguen in 1895 devised a preparation in which aortic outflow was assumed to equal coronary flow. Later workers

notably Markwalder and Starling (1913) thought that the results obtained were too low. However, a fresh impetus to such studies was given by the introduction by Moravitz and Zahn (1914) of the double lumen catheter for sampling coronary sinus blood uncontaminated by right atrial blood. This was an important step forward because although advances were being made in coronary physiology using the non-working isolated perfused heart preparation introduced by Langendorf in 1899 the Moravitz cannula could be used in the heart beating in situ. Since then many modifications in the heart lung preparations have been made and have contributed to the pool of knowledge.

Subsequently newer methods have involved the use of bubble flow meters and electromagnetic recording devices. Until recently these have necessitated the use of open chest preparations but the introduction of the nitrous oxide technique of measuring blood flow which simply required coronary sinus catheterisation made it possible to obtain a measure of coronary blood flow not only in the intact dog but also in the human subject.

Recent interest in the possibility of direct surgical attack on the coronary vessels has given fresh impetus to the problems of myocardial blood flow and metabolism.

METHODS OF MEASURING CORONARY BLOOD FLOW

Practically all of the methods which have been devised for measuring blood flow in general have been utilised in the coronary arteries.

Venous outflow collection and drop recording techniques have been generally unsuitable for work in this field. Bubble flowmeters as introduced by Soskin, et.al. (1934) have been used in the coronary arteries as also methods using the Thermostromuhr.

Blood tissue exchange methods including the nitrous oxide technique have been of great value in the last twenty years or so. Radioactive substances can be easily adapted to this type of technique (vide infra).

Test substance dilution methods using dyes such as indocyanine green can also be used but this method will only give the sinus outflow values.

When considering the usefulness of the various methods used in studying the coronary circulation consideration must be given as to whether phasic flow or mean flow is to be studied.

Phasic flow methods were devised with the primary object of studying factors in coronary flow which were too rapid to allow for consideration by a method which would only give mean flow. These

methods record the instantaneous flow at the point of insertion into the blood vessel.

Some of these methods included estimation of the phasic difference between the central and peripheral coronary pressure curves during a cardiac cycle (Gregg, 1937), bristle flowmeters, and differential pressure manometers. By far the most successful method has been, however, the introduction of electromagnetic flowmeters. These are of two basic types - square wave and sine wave. The square wave type has provided much useful information from experiments in open chest dogs but are of necessity fairly bulky and cannot be used in closed chest experiments. On the contrary the sine wave type has been miniaturized and can be applied to the coronary arteries in dogs over long periods. This flowmeter has provided much of the basic information which is currently available about the phasic behaviour of coronary flow in different experimental situations.

In the investigation of mean flow the graduated collection of outflow has little place in physiological experimentation nowadays. Bubble flowmeters and rotameters have been of great value in heart lung preparations as also have been techniques using the principle of heat clearance (Grayson and Mandel, 1961).

In man most of the methods have used variations of the Fick principle. The nitrous oxide technique used particularly by Ming

(1951) and Gorlin, et.al. (1959) has furnished the majority of information about the human coronary situation. This technique was developed from that used by Kety and Schmidt in 1945 to measure cerebral blood flow by the inhalation of metabolically inactive foreign gas, i.e. 15% nitrous oxide. The principles of this technique are as follows: Nitrous oxide dissolves in heart muscle in a nearly one-to-one ratio with the blood. The myocardial concentration of nitrous oxide can be determined by measuring the coronary venous concentration after 10 minutes of inhalation. At this point the tissues are saturated with gas: the myocardium and coronary venous blood are in virtual equilibrium. An arterio-venous difference is measured during the period of saturation and this is integrated by taking multiple timed samples. The A-V difference is divided into the nitrous oxide uptake per 100 grams to derive the flow. It was found by Gregg, et.al. (1951) that in dogs a ± 12.4% correlation of flow with rotameter measurements could be achieved.

Radio-Isotope Techniques

Holting, et.al. (1958) and Love and Burch (1958) used rubidium 86 uptake to determine coronary flow but this method has been shown to be very inaccurate. Sevelius and Johnson (1959) attempted to measure flow from analysis of a standard radiocardiogram after injection of I^{131} albumin. This too is inaccurate and cannot be

recommended for this type of work.

A recent modification has been the technique of coincidence counting of the positron emitter ^{84}rubidium. In this technique activity in the heart chambers can be corrected for by simultaneous injection of a non diffusible tracer such as ^{131}I human serum albumin.

It was decided that an inert gas clearance technique using ^{133}Xenon was most suitable and this technique is described in the following chapter.

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Chapter 2

THE INERT GAS CLEARANCE METHOD OF MEASURING MYOCARDIAL BLOOD FLOW

The use of solutions of radioactive gases in saline for the measurement of coronary blood flow was first described by Herd et.al. in 1962. Further work on this was done by Cohen et.al. in 1964 and Ross et.al. also in 1964.

The basic principle is that radioactive gas is injected into the coronary artery and this is carried to the heart and diffuses rapidly from the capillaries throughout its substance. Thereafter arterial blood containing none of the radioactive gas removes the isotope from the tissues of the heart and since it is highly diffusible its rate of removal is determined by the capillary blood flow. Thus the clearance of the radioactive gas from the heart is a direct method for measuring myocardial blood flow.

It is important to remember that although this method is in principle an indicator dilution method, the indicator does not remain in the vascular compartment as happens for instance in the measurement of cardiac output but it diffuses throughout the tissue space. The removal of the indicator by progressive re-equilibration with fresh capillary blood gives the essential measurement - that of the clearance of the isotope from the myocardium.

The basic formal mathematical analysis is now presented but before this is discussed the following assumptions are understood.

(1) The method assumes that the isotope diffuses rapidly throughout the tissue supplied by the artery in concentrations determined by the partition co-efficient (λ) between myocardium and blood.

$$\therefore \lambda = \frac{\text{concentration of isotope in myocardium}}{\text{concentration of isotope in blood}}$$

(2) Partition equilibrium occurs within the capillary transit time.

(3) The detector over the heart gives a count rate proportional to the radioactivity in the cardiac tissues.

(4) The arterial blood during the measurement contains no isotope.

(5) A single system is being measured in which capillary flow over the time of detection is constant.

The radioactive gas originally used in this type of experiment was ^{85}Krypton but for reasons discussed later ^{133}Xenon was chosen.

Calculation of myocardial blood flow

A single bolus of radioactive gas is introduced into the artery.

Now let Ca = concentration of Xenon in the artery.

Cv = concentration of Xenon in the vein.

Cm = concentration of Xenon in the myocardium

Qm = mass of Xenon in the myocardium

Vm = volume of myocardium.

F = flow of blood.

The rate of change of the amount of gas present in the myocardium is expressed in terms of flow by the Fick principle.

$$\frac{d\ Qm}{dt} = F\ (Ca - Cv) \qquad \ldots\ldots (1)$$

After passage of bolus, Ca = 0

$$\therefore \quad \frac{d\ Qm}{dt} = -F.Cv \qquad \ldots\ldots (2)$$

But at equilibrium $\lambda = \frac{Cm}{Cv}$ $\therefore$ $Cv = \frac{Cm}{\lambda}$ $\ldots\ldots (3)$

$$Cm = \frac{Qm}{Vm} \therefore Cv = \frac{Qm}{Vm} \qquad \ldots\ldots (4)$$

$\therefore$ Substituting (4) in (2) $\frac{d\ Qm}{dt} = \frac{-F.Qm}{\lambda Vm}$ $\ldots\ldots (5)$

(5) is a standard differential equation whose general solution is

$$Qm = Qm\ (0)\ e^{-kt} \qquad \ldots\ldots (6)$$

(6) is the exponential decay expression in which Qm (0) is the value of Qm at the initial part (t = 0) of the measured decay. In the particular solution the clearance rate constant is

$$k = \frac{F}{\lambda Vm}$$

$$\therefore \quad \frac{F}{Vm} = k\lambda \qquad \ldots\ldots (7)$$

As the partition coefficient is known the expression means that flow per unit volume can be found when the only unknown -k- is calculated.

Calculation of k

K is not measured directly but is calculated from the time required for the exponentially decaying Qm to fall from any value to half that value. This is calculated thus:

In the expression $Qm = Qm\ (0)\ e^{-kt}$ - if the time required for Qm (0) to fall to half its value is $t_{\frac{1}{2}}$ then

$$\frac{Qm}{2} = Qm\ e^{-kt_{\frac{1}{2}}}$$

$$\therefore \frac{1}{2} = e^{-kt_{\frac{1}{2}}}$$

$$\therefore \frac{1}{2} = \frac{1}{e^{kt_{\frac{1}{2}}}}$$

$$\therefore e^{kt_{\frac{1}{2}}} = 2$$

$$\therefore kt_{\frac{1}{2}} = \log_e 2$$

$$\therefore k = \frac{\log_e 2}{t_{\frac{1}{2}}}$$

As $\log_e 2$ is 0.69315 k is therefore known when the half time $t_{\frac{1}{2}}$ (in minutes) is found. This is done in the standard manner by plotting the points of the clearance curve on semilogarithmic paper. From the straight line which is plotted the half time is derived and k is calculated.

Equation (7) can now be written thus:

$$F = k \lambda Vm \qquad \ldots\ldots (8)$$

If the volume of myocardium chosen is 100 ml. then

$$F = k \lambda 100 \text{ ml./min./100 ml.} \qquad \ldots\ldots (9)$$

As it is customary to express flow in ml./min./100 g. of tissue the dimensions of equation (9) are transformed thus:

$$\text{Flow} = k\lambda \frac{100}{\rho} \text{ ml./min./100 g.} \quad \ldots\ldots (10)$$

where ρ = density of myocardium

As ρ = 1.05 mg./ml. (Herd et.al. 1962) and

λ = 0.72 (Conn 1961).

Then myocardial blood flow = $\frac{k \times 0.72}{1.05} \times 100$ ml./min./100 g. $\ldots\ldots (11)$

$\therefore$ Myocardial blood flow = 68.5 k ml./min./100 g.

Figure 2 shows a typical clearance curve obtained with this method and the derivation of the blood flow therefrom.

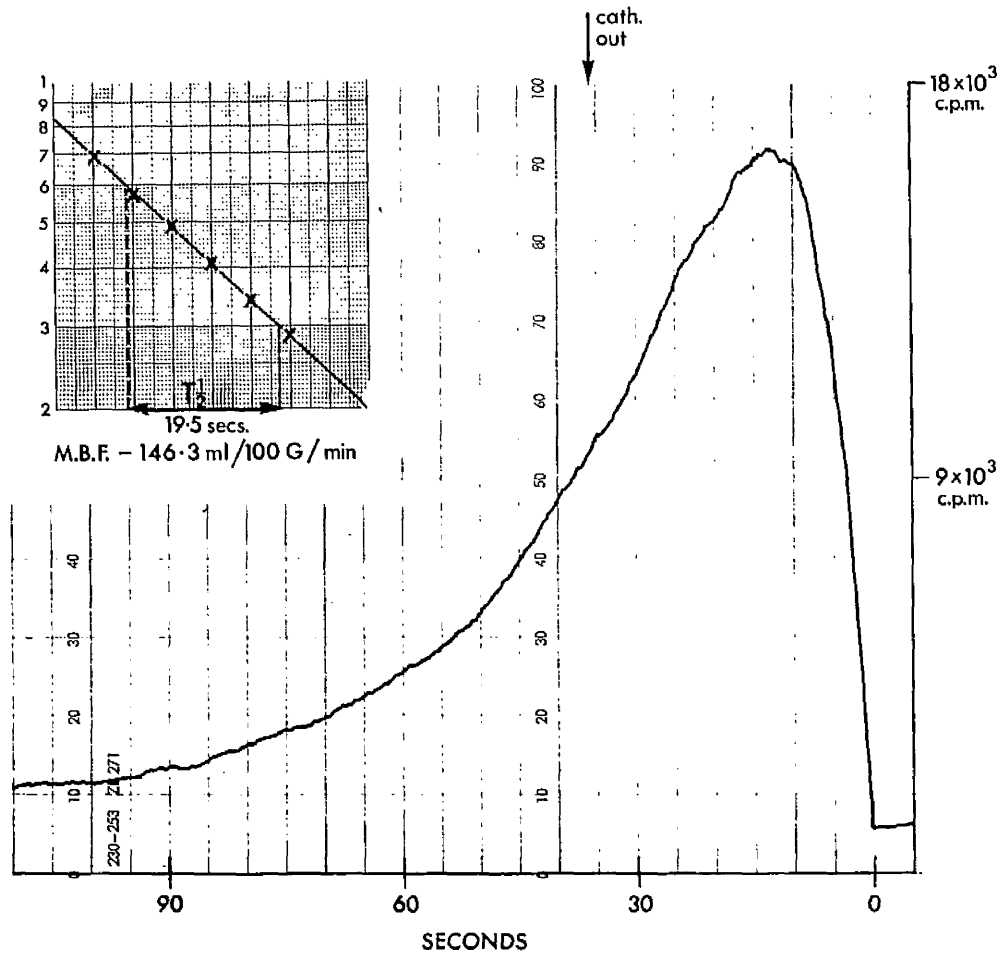

Figure 2: This shows a typical clearance curve obtained by this technique of measuring myocardial blood flow. The Inset shows the transference of the initial slope of the curve to semi-logarithmic paper and the derivation of the half-time from the slope of the straight line produced.

DISCUSSION OF THEORY

The first obvious difficulty with this type of theoretical approach is that it only deals with flow per unit volume or mass of tissue and not absolute flow. This can be dealt with in experimental situations by a technique which allows for calculation of the absolute mass of tissue perfused by the artery used in the injections. This can be done by injection of dyes or of barium and the actual tissue involved weighed. Absolute flows can then be derived. In experimental work where the degree of change in flow is important then flows found in this manner can be used and the percentage change easily calculated.

The second difficulty is that there is an obvious discrepancy between the theoretical predicted curves and the actual curves recorded. Inspection of a typical curve shows that it is not in the form of a single term exponential and analysis on semilogarithmic paper indicates that, in fact, the majority of experimental curves approximate to a bi-exponential form.

The early workers with this technique (Ross, et.al. 1964) devised an experimental approach by which flows to a coronary artery could be measured by rotameter in a heart-lung preparation and simultaneously estimated by the radioactivity gas clearance technique. The correlation coefficient found for their experiments was 0.99.

Zierler (1965) in a review of the theoretical derivation of

the equations for measuring blood flow by external monitoring of radio-isotopes, pointed out that in such equations the likelihood of the curve being truly exponential was much increased if the flow per unit volume was sufficiently rapid. Lassen (1967) confirmed this and also suggested that of all the areas in which this type of technique had been used to measure flow, the myocardium was by far the most convincing from a theoretical basis.

Zierler further discussed the analysis of the curve described and by a theoretical presentation of the functions of transit times was able to show that

$$\frac{F}{V} = \frac{go}{\int_0^\infty q(t)dt} = \frac{\text{peak (or zero time) value}}{\text{area}}$$

and in this equation it is not necessary to assume that equilibrium between blood and tissue exists. It is therefore theoretically possible to calculate the flow by two separate methods (a) the semilogarithmic replot and derivation of half-time ($t\frac{1}{2}$), (b) the peak height over area method.

Zierler showed that in the given individual instances the first method overestimated the second by 17%, although it is possible to underestimate or even equal the other value. Obviously the nearer that the semilogarithmic replot approximates to a straight line, the likelier is that these two values will agree. To deal with the possible effects of this discrepancy in calculation Rees and Redding (1967) in a long series compared results achieved by these two

different methods of calculation. In individual cases the percentage difference remained intact the correlation coefficient being very high indeed. In the work done in our own laboratories during the initial development of this method, changes in blood flow produced by various manoeuvres were estimated by both techniques and the percentage changes recorded were identical.

The diffusion equilibria between capillary blood and tissues is an important factor but the assumption of continuous diffusion equilibria is reasonable for highly diffusible indicators such as heat, water or Xenon (Bassingthwaite et.al. 1968).

One factor of importance is the high degree of solubility of Xenon in fat. It soon became obvious in our early series of experiments that the slope of the curve and the degree of residual radioactivity varied directly with the amount of fat in the myocardium being perfused. This problem of lipid solubility had been examined by Friesinger (1968) who was able to demonstrate that the residual high level of activity was due to the persistence of Xenon in fat. This was done by a technique which involved serial autoradiographs.

Bassingthwaite, et.al. (1968) likewise devised an experiment by which flow estimated by this technique was measured and compared to actual flow. The highest correlation was achieved using the standard mono-exponential analysis of the curve produced by the

clearance of ^{133}Xenon.

The partition coefficient (λ) for Xenon between myocardium and blood was taken as 0.72, a fact worked out in detail by Conn (1961). As our work involved greater than atmospheric pressures it was important to check that this partition coefficient did not vary with differing pressures. There seemed to be no physical reason why this should happen but it was checked by producing Xenon curves in some experimental animals at differing atmospheric pressures while keeping the inspired oxygen tensions at a constant level. This showed no difference in peak height, shape of curve or derived blood flow and it was assumed that the Xenon diffused equally at all the working pressures.

PRACTICAL DETAILS OF THE METHOD

The basic experimental set up is as shown in the diagram (Figure 3) and the photograph (Figure 4). Mongrel dogs were anaesthetised with thiopentone sodium given intravenously, intubated and anaesthesia was then maintained by trichlorethylene using a Tritec vapouriser. Respiration was maintained by means of a Starling pump and spontaneous respiration abolished using succinylscoline. Depth of respiration was usually adjusted to keep the arterial PCO_2 between 35 and 45 mm. of mercury.

Using the left common carotid artery a Sones type of coronary artery catheter was inserted under radiographic control into one of the coronary arteries. These catheters have a tip which is tapered down to size 5 (French Gauge) and the tip was placed just inside the mouth of the artery. In this position there should be no obstruction to flow and this was confirmed by withdrawing the catheter during the inscription of a washout curve, and no interruption of the curve ensued (See Figure 2, page 21.)

CHOICE OF RADIO-ACTIVE GAS

Although both ^{85}Krypton and ^{133}Xenon had been used in earlier studies with this method, it was decided to use ^{133}Xenon. Both gases have short biological half lives and it was found both for ^{85}Krypton (Chidsey, et.al. 1959) and for ^{133}Xenon (Ross, et.al. 1964)

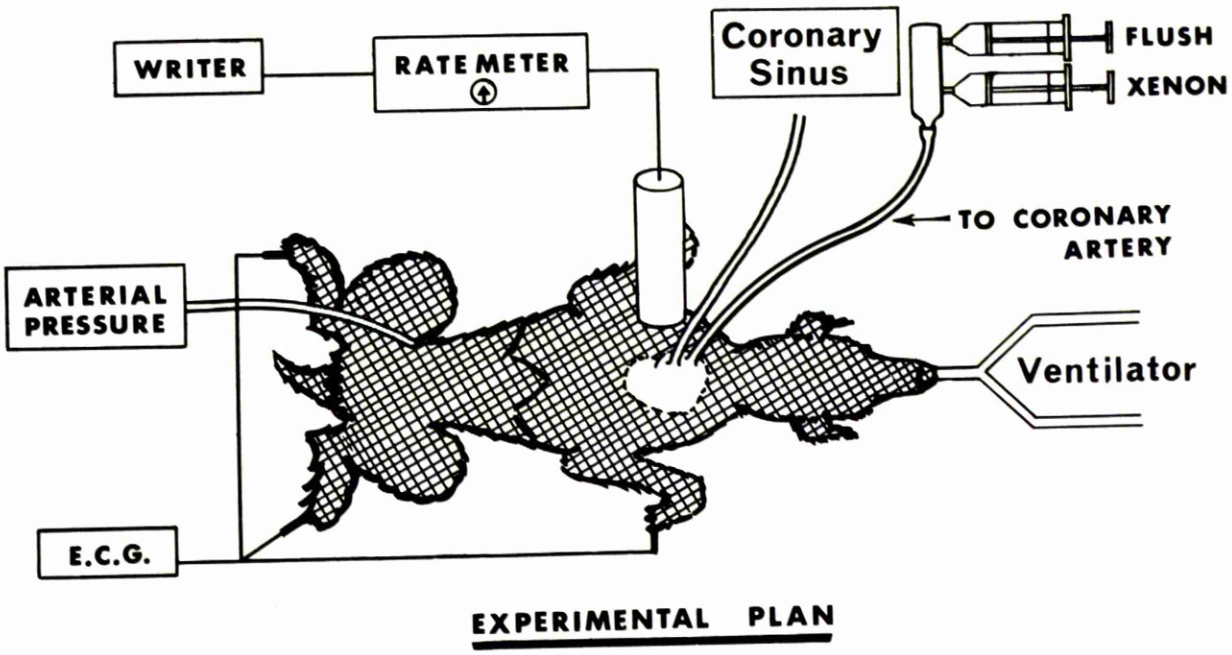

Figure 3: Diagram of experimental plan.

Figure 4: The experimental set up is shown. The scintillation counter is in position over the chest wall. The coronary artery and sinus catheters are shown at the neck. Radio-active counting equipment is in the foreground, and upper left there is the image intensifier.

that only 5% of an injected intravenous dose reached the systemic circulation. The longer physical half life of Krypton (10.27 years) and its higher gamma energy emission (540 KEV) make it a greater radiation hazard than Xenon which was therefore selected for use.

^{133}Xenon has the following characteristics:

Atomic Number	54
Mass Number	133
Half life	5.27 days
Radiation	Beta and gamma
Beta	0.110 MEV
Gamma	Main component 81 KEV (35.5%)

As supplied from the Radio Chemical Centre at Amersham Xenon gave normally 1 mc/ml. (dissolved in sterile isotonic saline).

To give satisfactory counting rates it was found that 0.5 ml. of the solution was the dose required. Experiments initially performed showed that satisfactory bolus injection could be achieved by flushing this dose of Xenon through the coronary artery catheter with 2.5 - 3 mls. of saline. It was also discovered that using other substances to flush the Xenon into the catheter, e.g. 5% dextrose, warm saline or blood had no effect on the curves produced and isotonic saline at room temperature was therefore used.

A detector using a 2" sodium iodide crystal was positioned over the heart at a spot which gave satisfactory counting rates and

curves. The ideal position for any one animal could quickly be found. The detector utilised narrow angle collimation and impulses fed through a pulse height analyser to a ratemeter and from there to a writer. The equipment used was an Ekco radioactive counting system and a Servoscribe. The pulse height analyser gate was set for the appropriate gamma energy and the ratemeter normally set at 3 second rate constant. The type of curve produced is shown in Figure 2 and the flow derived as previously described.

As most of the designed experimental work involved varying the ambient pressure, a pressure vessel large enough to accommodate all the appropriate equipment was necessary. Such a vessel is incorporated in the Hyperbaric Unit at Glasgow Western Infirmary. As can be seen from the picture of the interior (Figure 5) this pressure chamber was designed for use as an operating theatre (and it is in fact currently being so used). There is therefore ample space for working, installation of instruments, etc. and the experiments could be designed to include all the types of equipment which were used initially at normal pressure.

This pressure chamber is designed to compress the air inside the vessel and it is important to remember that in the larger type of pressure vessel the compressing gas is air not oxygen. This is in contrast to the situation in the smaller pressure chambers designed for clinical use "the one-man pressure chamber" in which the compressing gas is oxygen.

The terminology used to record the variation in pressure can be confusing and the various types of nomenclature are set out in the following table (Table 1) which shows the changes in pressure when descending below sea level.

In the Glasgow pressure chamber, the maximum pressure available is three atmospheres absolute (3 ATA). It was decided that our experiments should be limited to two atmospheres absolute and the

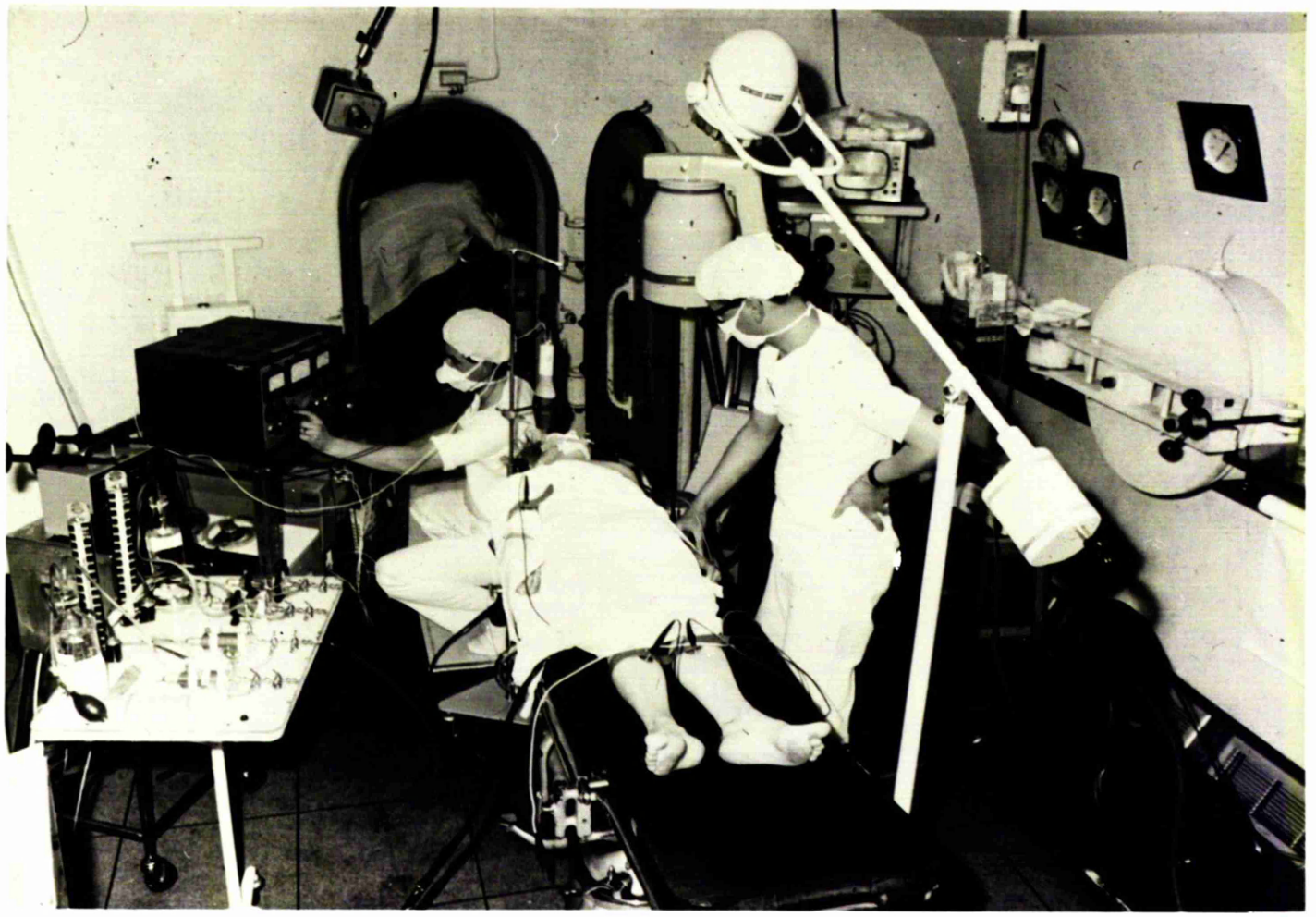

Figure 5: This photograph shows the interior of the pressure chamber and demonstrates the room available for both personnel and equipment.

Table 1

	Atmospheres Absolute (ATA)	Barometric Pressure		Gauge Pressure lb./sq.in.
		mm. Hg.	lb./sq.in.	
Sea level	1	760	14.7	0
33 ft. depth	2	1520	29.4	14.7
66 ft. depth	3	2280	44.1	29.4

reasons are:-

(a) Two atmospheres absolute (2ATA) could give in theory an arterial PO_2 approaching 1200 mm.Hg. if 100% oxygen is the inspired gas. It was felt that this level of oxygen tension would be sufficient for our investigative purposes.

(b) In the clinical practice of the Hyperbaric Unit most treatment was carried out at two atmospheres.

(c) The question of hazards to staff.

Working at high atmospheric pressure is known to have potentially harmful effects. The two most important are the different types of Caisson disease ("the bends") and aseptic bone necrosis. Experience of the hazards experienced by commercial concerns and

also other clinical and experimental hyperbaric facilities has shown that the decompression schedules must be rigidly adhered to if trouble was to be avoided. However, if the working pressure does not exceed two atmospheres absolute then the decompression time required is very short - and furthermore no case of bone necrosis has yet been recorded if the working pressure was not in excess of two atmospheres absolute. Bone surveys have been conducted on the Western Infirmary hyperbaric personnel since the start of the programme and no cases of bone necrosis have been found, nor have there been any cases of decompression sickness (Davidson and Ledingham, 1969). Personal experience of possible hazards has been one mild episode of otitic barotrauma. This occurred when going to pressure with a mild head cold but settled with no treatment in a few days.

The use of ^{133}Xenon does not by itself present a significant radiological hazard. This is because Xenon is quickly cleared from the general circulation by passage through the lungs and is excreted to atmosphere. If the expired air containing Xenon can be vented to the atmosphere it is quickly diluted to insignificant levels. When working in the pressure chamber the expired air could easily be led out through the exhaust valve to atmosphere and in fact there was no difficulty with build up of background radiation. The recording apparatus and the radioactive counting equipment was tested at pressure and all were found to work

satisfactorily.

The radiological screening equipment was only used occasionally at pressure as most of the screening could be accomplished before going to greater than atmospheric pressure but on the occasions when it was used at pressure it functioned satisfactorily.

One last hazard was the possibility of an increased fire risk at pressure. In the large pressure chamber which was being used for these experiments the compressing gas is air and the theoretical increase of fire risk at two atmospheres is very slight. In spite of this the chamber is constructed with fire risk in mind and all appropriate safety switchgear etc. is incorporated. In practice there were no hazards from fire or explosion.

EVALUATION OF TECHNIQUE

Preliminary studies of the technique were performed on 20 mongrel dogs weighing from 9.8 to 21 kg.

The anaesthetic selected for this study was trichlorethylene. The reasons for this choice were that much previous work had been done with trichlorethylene anaesthesia at hyperbaric pressures and the behaviour of trichlorethylene from the anaesthetic point of view was well documented, (Jacobson et.al., 1963; McDowall et.al, 1964). It also provided an anaesthetic which would keep an experimental preparation stable over a period of many hours with only minor changes in concentration.

A total of 210 measurements of flow were made. The average period of experiment was three hours and Figure 6 shows a typical pattern of behaviour in one dog. After an initial high peak of flow after induction and intubation the flow settled within 30 minutes to an acceptably stable state.

Blood pressure and heart rate were obviously important in this context and the mean results are presented likewise in graphical form (Figure 7).

The total reduction in blood pressure over the three hours was less than 10% and heart rate did not vary significantly when the stable state had been reached.

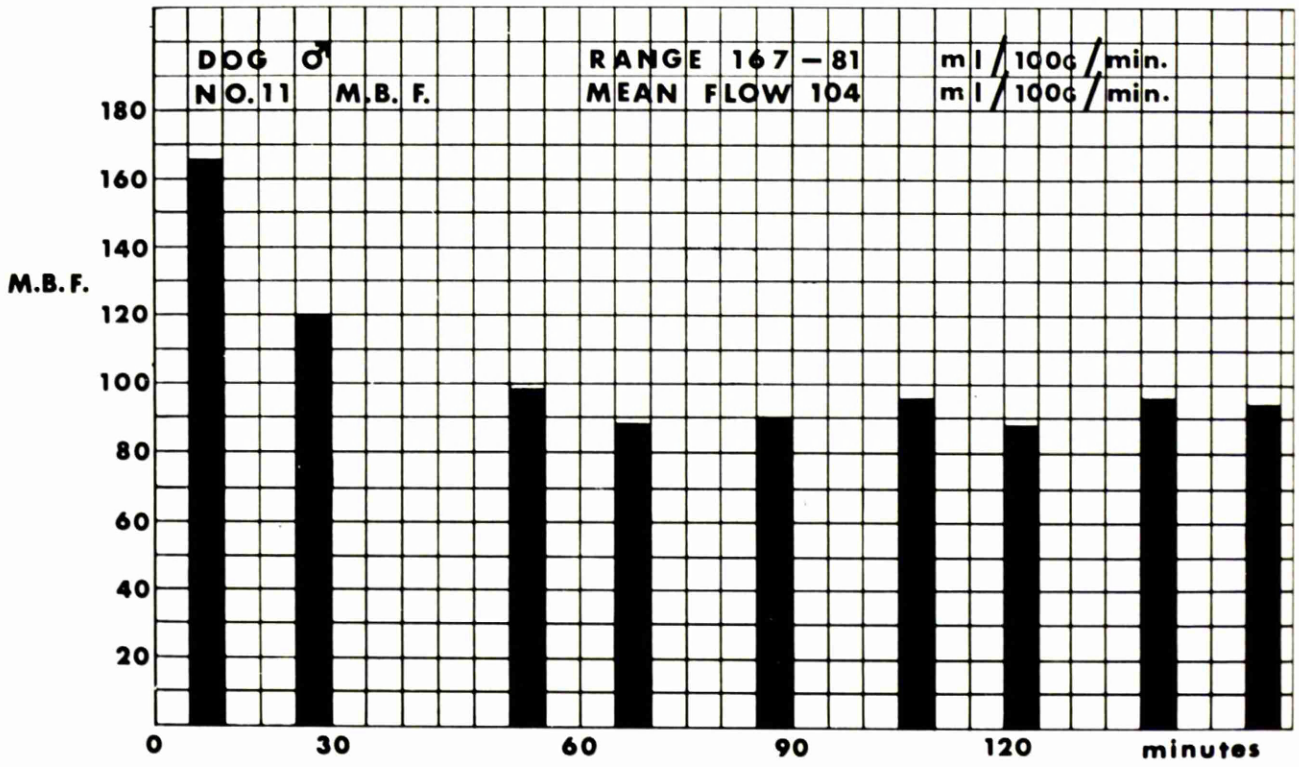

Figure 6: This histogram shows the behaviour of myocardial flow in one animal over a 3½ hour period. After the initial peak, flow quickly settles to a stable state.

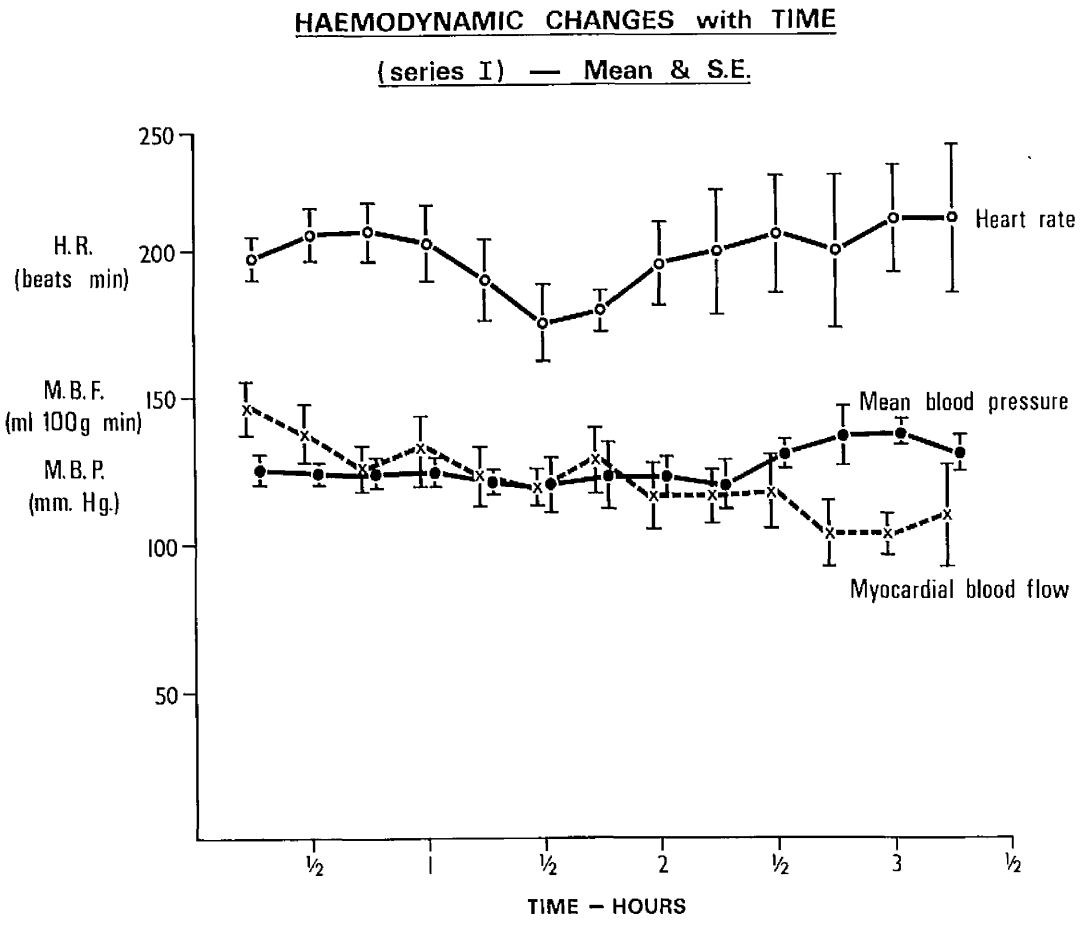

Figure 7: This diagram presents the changes in heart rate, mean blood pressure and myocardial blood flow over the 3½ hour period.

To assess the reproducibility of the technique of measurement paired runs were undertaken throughout the series of 210 measurements. With the Xenon clearance technique it is possible to repeat runs within three to four minutes. Pairs were selected in which the heart rate and blood pressure were identical and it was found that the variation between the pairs was always less than $\pm$ 4% of the mean flow. This degree of reproducibility was considered acceptable. In these flows the range recorded was 70 - 210 ml. per 100 g. per minute and the mean flow was 126 ml. per 100 g. per minute.

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Chapter 3

CHANGES IN MYOCARDIAL BLOOD FLOW WITH EXPOSURE TO HIGH PARTIAL PRESSURES OF OXYGEN

As the initial research interest had lain in the investigation of the responses to myocardial blood flow to high partial pressures of oxygen the first set of experiments were designed to elucidate this point.

EXPERIMENTAL METHOD

The experiments were all performed in the pressure chamber at the Western Infirmary, Glasgow. Mongrel dogs with weights varying from 9-23 kgs. were used. Anaesthesia was induced by intravenous sodium thiopentone, and maintained with trichlorethylene given via a Tritec vaporiser. Respiration was maintained by means of a Starling pump and spontaneous respiration was abolished with succinylcholine. Tidal volumes were controlled by means of repeated blood gas estimations and the arterial PCO_2 was kept between 35 and 45 mm. Hg. Temperature was controlled by means of a heating cage.

Heparin 2,500 units was given every two hours. E.C.G. was recorded by means of needle electrodes. Cannulae were inserted into the femoral artery and vein. Pressure at the aorta was measured - systolic, diastolic and mean arterial pressure being

recorded on a Mingograf 81 recorder.

With the aid of a Siemens 5" image intensifier the left coronary artery was cannulated usually with a Sones No. 7 catheter. The commonest site was the left circumflex coronary artery although left anterior descending was occasionally used. The coronary sinus was cannulated using a catheter manufactured from Teflon. At the end of the experiment the catheter was withdrawn during a curve to ensure that no obstruction to flow had occurred.

To obviate any differences which may have been due to the effect of varying ambient pressures all the experiments were conducted at two atmospheres absolute. Variation of the arterial PO_2 was achieved by first using a mixture of oxygen and nitrogen in proportions which would give an arterial PO_2 of between 90 and 100 mm.Hg. This mixture of gases was easily controlled by using a paramagnetic oxygen analyser (Servomex). Due attention was paid to the daily blood-gas difference in the oxygen electrode system and allowance made when calculating the desired PO_2. This method of working produced what is subsequently referred to as "air equivalent" i.e., a combination of oxygen and nitrogen is found which at the appropriate ambient pressure will give an arterial oxygen tension of between 90 and 100 mm.Hg. When the inspired gas was changed to 100% oxygen the arterial PO_2 obtained averaged 1000 mm.Hg. Mean arterial blood pressure was obtained by using

the electronic integrator in the recording machine and the heart rate counted by the E.C.G. over a 30 second period. The experimental procedure was to measure heart rate, blood pressure and myocardial blood flow firstly on air equivalent and then after a rapid change to breathing 100% oxygen.

RESULTS

The overall results of this phase of the experiment are here presented. 34 dogs were studied.

Table 2

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR and O_2 at 2 ATA (34 DOGS)

	Heart Rate per min.	Mean Arterial Blood Pressure mm.Hg.	Myocardial Blood Flow ml/100g/min.
AIR = Mean and S.E.	161 ± 4.84	116 ± 2.89	114 ± 4.28 p 0.001
O_2 Mean and S.E.	156 ± 5.38	114 ± 4.28	85 ± 3.51

COMMENT

These results show a reduction in blood flow of 25%. Heart rate and mean blood pressure remain virtually unaffected. Statistical analysis by means of the unpaired t test shows that the reduction in blood flow is highly significant $p<0.001$.

An interesting finding was that examination of the heart rate and blood pressure by the paired t test gave significant results (heart rate $p< 0.025$, mean blood pressure $p< 0.01$) indicating that the direction of change was significantly that of a reduction in each case although obviously the magnitude is not of statistical or of physiological significance. Studies of the electrocardiographic records taken during these experiments showed no changes in rhythm, atrio-ventricular conduction or QRS configuration.

As it had been established that the change from air equivalent to oxygen was associated with a reduction in myocardial flow it was decided to examine the changes in the reverse direction, i.e. oxygen to air equivalent, to see if any significant difference existed.

METHOD

The basic procedure was as in the first experiment but two groups of dogs were examined. In the first 12 dogs changes in

blood flow were examined on changing the inspired gases in this order: air equivalent : 100% oxygen : air equivalent : 100% oxygen. In the second group of 11 dogs the sequence was reversed. The results are presented in the following histogram. (Figure 8).

COMMENT

The pattern of response is similar in both series. The overall mean reduction of flow achieved was in this case 21%.

Barbiturate Anaesthesia

In a final experiment four dogs were examined by this basic procedure of changing the inspired oxygen content over a short period of time with one main difference. The experiment was conducted solely under barbiturate anaesthesia - in this case sodium thiopentone.

The mean results are presented in the following table (Table 3).

Although the series is small the reduction in myocardial blood flow is again significant in this case being 35%. The possibility of differing influences of various anaesthetic agents was not pursued.

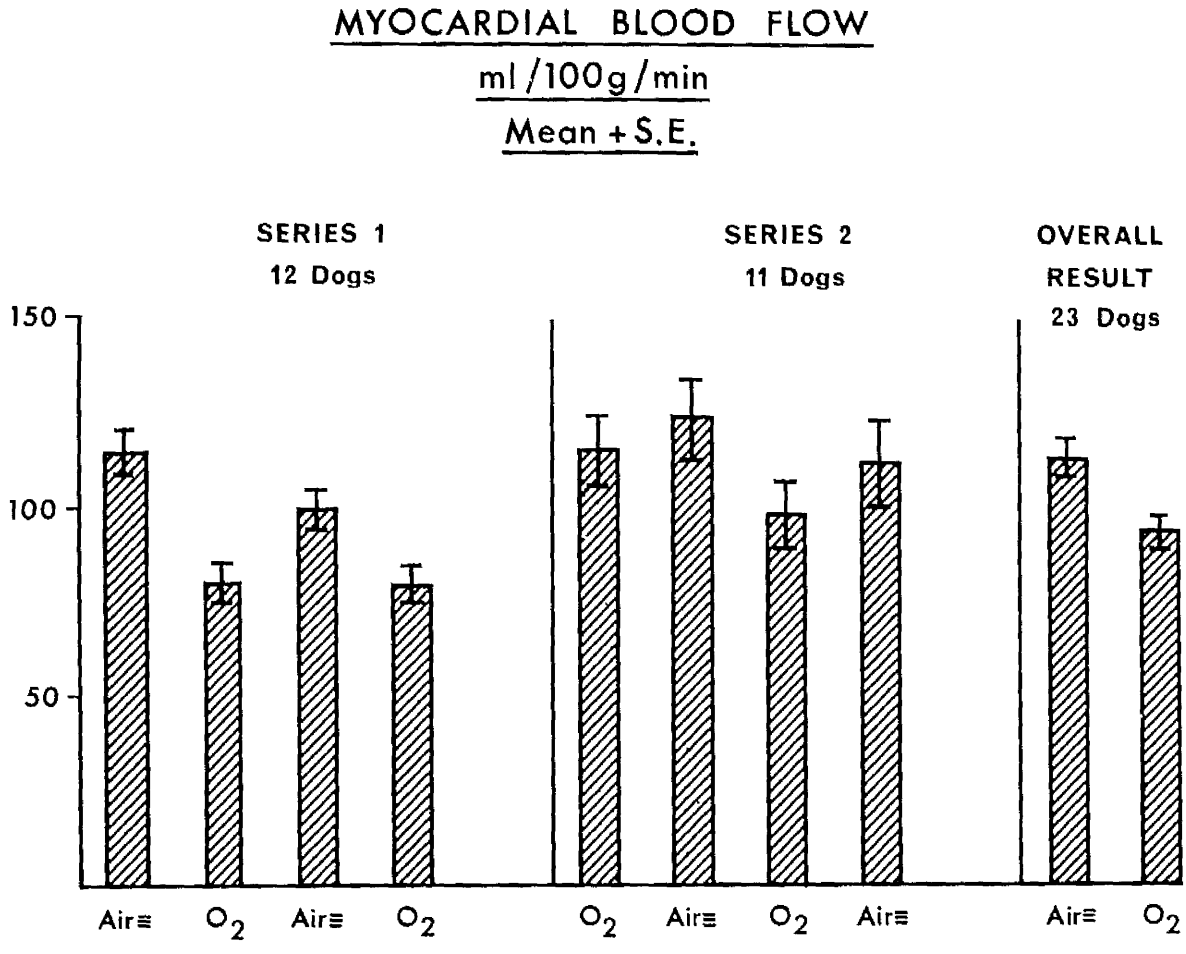

Figure 8: This histogram shows the results on changing from air equivalent to oxygen and then when the sequence is reversed. The final columns show the overall results in 23 dogs.

Table 3

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR and O_2 at 2 ATA (4 DOGS)

	Heart Rate per min.	Mean Arterial Blood Pressure mm.Hg.	Myocardial Blood Flow ml/100g/min.
Air equivalent	166	97	92
100% oxygen	156	80	66

DISCUSSION

It has been known for some time that hypoxaemia is a potent stimulus to an increase in myocardial blood flow and it has long been assumed that an increase in arterial oxygen tension would possibly result in a reduction of myocardial blood flow. Early workers in this field reported a decrease in myocardial blood flow with increasing concentrations of oxygen at normal environmental pressure. Sobol, et.al. (1962) in the open chest dog increased the arterial blood oxygen content from an average of 18.1 to 20.9 volumes per cent by ventilating with 100% oxygen and found a decrease of 22% in coronary sinus outflow.

Eckenhoff, et.al. (1947) reported similar findings with 100%

oxygen at normal ambient pressures while more recently Daniell and Bagwell (1968) again with open chest dog preparations found that abrupt changes in inspired oxygen concentrations (from 25-100%) were associated with consistent reductions in flow.

As far as increases in arterial oxygen tension with hyperbaric pressures are concerned, the first report on this was by Meijne and Straub (1966). They used the technique of measuring coronary sinus outflow to estimate myocardial blood flow and on ventilating with oxygen at one atmosphere absolute and three atmospheres absolute a progressive reduction in flow was observed. Weglicki, et.al. (1966) used a direct injection of Xenon into the myocardium as their technique of measuring myocardial flow. This was performed in the open chest dog at three atmospheres absolute and reductions of flow varying from 25 to 50% were recorded.

The results presented here, the first in a closed chest preparation, are in agreement with the trend observed by other investigators and it can be regarded as established that the myocardium responds to abrupt increases in oxygen tension to a level of 1000 mm.Hg. with a decrease in myocardial blood flow. As there is no significant change in blood pressure or heart rate this implies a marked increase in myocardial vascular resistance. This is consistent with the trend found in many other areas of the body.

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Chapter 4

INVESTIGATION OF THE MECHANISM OF BLOOD FLOW REDUCTION ON EXPOSURE TO HIGH PARTIAL PRESSURES OF OXYGEN

When it became clear that the response to high partial pressures of oxygen was a consistent reduction in flow an investigation into the mechanisms of this change was planned. From the point of view of modifying this response by the use of drugs it seemed important to exclude a nervous reflex as the mechanism although it was considered that this was unlikely to be the case.

The widespread sympathetic innervation of the heart was known although the extent of vagal innervation remained in doubt. Jose (1966) had investigated cardiovascular responses in what was called "the pharmacologically denervated heart". By this was meant total blockade of the heart by using atropine and propranolol, i.e. a combination of vagal and beta sympathetic blockade. Accordingly the first experiment was concerned with observations of the myocardial flow response to oxygen before and after injection of these drugs. Later it became clear that the effect of alpha blockade should be examined and this was performed using phenoxybenzamine. The final experiment investigated total adrenergic neurone blockade by the use of bretylium tosylate.

Effect of Atropine and Propranolol

The basic experimental set-up was as previously described. The parameters studied were mean arterial blood pressure, heart rate and myocardial blood flow. Changes in the inspired gases were arranged in the following order.

Atropine + Propranolol ↓	
O_2 : Air : O_2 : Air=	Air= : O_2 : Air=

Atropine 0.04 mg./kg. and propranolol 0.2 mg./kg. were given intravenously at the indicated points. The results are presented in the following table. (Table 4).

Table 4

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
EFFECT OF ATROPINE AND PROPRANOLOL

7 DOGS AT 2 ATA

Mean and S.E.

	Heart rate per min.	Mean Blood Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
100% O_2	175 ± 13	125 ± 3	104 ± 14
Air equivalent	180 ± 12	122 ± 5	118 ± 12
100% O_2	185 ± 10	126 ± 3	96 ± 11
Air equivalent	177 ± 13	118 ± 3	113 ± 8
	Atropine + Propranolol		
Air equivalent	150 ± 5	118 ± 5	78 ± 2
100% O_2	155 ± 11	113 ± 5	61 ± 6
Air equivalent	159 ± 8	116 ± 5	75 ± 9

COMMENT

This table shows the changes previously recorded in blood flow on changing from oxygen to air and from air to oxygen. After the injection of atropine and propranolol, heart rate falls by 15%. There is a slight over-all fall in blood pressure and myocardial flow falls significantly (30%). The feature of interest is that

the pattern of change between air equivalent and 100% oxygen is preserved. The reduction in blood flow on changing to oxygen after atropine and propranolol is 20% compared to a mean reduction of 13% before injection of the drugs .

Effect of Alpha Adrenergic Blockade

Phenoxybenzamine, an alpha adrenergic blocking agent was investigated. The experiment was repeated and the change from air equivalent to 100% oxygen studied after injection of phenoxybenzamine (1 mg./kg.). Two dogs were studied and the relevant figures from the protocol of one dog are abstracted in the following table (Table 5).

TABLE 5

DOG 02628 MALE 21 Kg.
EFFECT OF PHENOXYBENZAMINE

Inspired Gas	Time	Heart Rate Beats/min.	Mean Arterial Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
Air ≡	1340	172	125	119
Air ≡	1350		Phenoxybenzamine 1 mg./kg.	
Air ≡	1400	212	110	114
Air ≡	1425	192	96	108
Air ≡	1440	184	95	110
	1445		Change to 100% Oxygen	
O_2	1455	176	97	85
O_2	1500	172	99	85
	1508		Change to Air ≡	
Air ≡	1515	176	97	108

COMMENT

Phenoxybenzamine induces a fall in blood pressure, but there is an increase in heart rate. Myocardial blood flow falls by 8%. In spite of the alpha blockade oxygen once again produces a reduction in myocardial blood flow which is reversed on changing back to air equivalent.

Effect of Adrenergic Blockade

Bretylium tosylate, an adrenergic blocking agent, was investigated. The basic experiment was repeated and the change from air equivalent to 100% oxygen repeated after intravenous injection of bretylium (10 mg./kg.). Two dogs were studied and gave similar results. The results from the protocol of one dog are presented in detail, (Table 6.)

Table 6

DOG 22268 MALE 20 kg.
EFFECT OF BRETYLIUM TOSYLATE
2 ATA

Inspired Gas	Time	Heart Rate Beats/min.	Mean Arterial Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
Air ≡	1405	184	105	114
Air ≡	1415		Bretylium Tosylate 10 mg./kg.	
Air ≡	1417	128	145	92
Air ≡	1426	136	132	89
Air ≡	1435	148	134	104
Air ≡	1500	142	110	89
	1505		Change to 100% Oxygen	
O_2	1515	124	110	77
O_2	1525	124	108	75
	1530		Change to Air ≡	
Air ≡	1540	130	110	92

COMMENT

After injection of bretylium there is a rise in mean arterial pressure although a fall in heart rate was noted. By the time adrenergic neurone blockade had been established myocardial blood

flow was at a level 21% below that of the control figure. On changing to 100% oxygen in this situation, a further fall in myocardial blood flow of 14% occurred and on changing to air equivalent this fall in flow was reversed.

DISCUSSION

The mechanism of the consistent reduction in flow encountered on exposure to high partial pressures of oxygen remains uncertain. The investigation reported in this section was designed to explore one possibility, i.e. reflex nervous vasoconstriction.

Until recently the mode of influence of the nervous system on the myocardial vasculature has remained far from clear. From anatomical work the extensive distribution of sympathetic nerve fibres to the heart is known (Tcheng, 1951), but doubts exist about the extent of vagal innervation. Berne (1964) reviewed the conflicting data and came to the conclusion that the vagi probably have little effect on the coronary vessels.

At the beginning of the study the first experiment was designed to use pharmacological denervation of the heart as suggested by Jose (1966). Following Ahlquist's work (1948) on the concept of specific adrenotropic receptors it had been thought that the beta receptors predominated in the coronary vasculature. If blockade could be established using both vagal

and beta adrenergic blocking agents, ie. atropine and propanolol then the heart could be isolated from reflex effects. The results presented clearly demonstrate that the effect of high partial pressures of oxygen on myocardial blood flow persisted after this manoeuvre had been effected.

The question of adrenergic receptors in the coronary circulation was reviewed by Parratt (1967 a). A considerable amount of evidence had accumulated to suggest that both types of adrenergic receptors, i.e. alpha and beta existed in the coronary vascular bed.

Parratt (1965) measuring myocardial blood flow by a thermo-electric method found that intravenous infusions of adrenaline in doses which had no effect on blood pressure or heart rate usually lowered myocardial vascular resistance: this was reversed after beta receptor blockade. Noradrenaline was slightly constrictor before beta blockade and subsequently markedly constrictor. These results suggested the presence of both alpha and beta receptors in the myocardial vascular bed. Zuberbuhler and Bohr (1965) using isolated smooth muscle strips from coronary arteries of varying sizes concluded that both types of receptor are present in the larger arteries, but that beta receptors predominate in the smooth muscle of the vessels of smaller diameter.

In view of this the experiment was repeated after alpha blockade

had been established with phenoxybenzamine, but the change produced by high arterial tensions of oxygen was still present.

To take the investigation further, the effect of bretylium tosylate was studied. This is an adrenergic neurone blocking drug which abolishes the responses to stimulation of post-ganglionic adrenergic nerves without abolishing responses to injected noradrenaline.

Parratt (1967 b) had found that bretylium initially raised arterial blood pressure and increased heart rate and myocardial blood flow. When adrenergic neurone blockade was complete arterial pressure, heart rate and myocardial flow were all reduced. In the experiment here reported the effect on blood pressure was similar to that reported by Parratt, but in fact a reduction in heart rate was achieved. Myocardial blood flow fell in a similar manner although contrary to Parratts findings there was no initial rise in flow. It may be that differences in type or depth of anaesthesia could account for some of these discrepancies. However, it is clear that adrenergic neurone blockade did not abolish the change observed when the inspired gas was varied from air equivalent to 100% oxygen. It is concluded that the mechanism of reduction in flow is not mediated through nervous reflexes.

The possibility of hormonal effects from cardiac muscle metabolites exists. If some concept of autoregulation is tenable

in the question of control of blood flow then local reflexes or the oxygen consumption of the myocardium must be considered. Oxygen consumption in this situation is obviously of great importance as it has been thought to be a major determinant of coronary blood flow (Berne, 1964). This has been studied and is discussed in a later chapter.

Review of the published work on the effects of oxygen on blood vessels makes it unlikely that the response is mediated through nervous reflexes. Many experimental observations indicate that vasoconstriction is the characteristic response to an increase in blood oxygen tension (Cusick, et.al., 1940, Dollery, et.al., 1964, Saltzman, et.al., 1965). Although the mechanism remains unclear it is felt that the suggestion of Guyton (1967) is most probable, i.e. that there is an intrinsic component in the coronary vessels which is directly responsive to arterial oxygen tension.

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Chapter 5

CHANGES IN MYOCARDIAL OXYGEN CONSUMPTION AND METABOLISM IN RESPONSE TO HIGH PARTIAL PRESSURES OF OXYGEN

It was decided to increase the scope of the investigation by estimating the oxygen consumption of the myocardium. To explore metabolic changes further simple parameters of carbohydrate metabolism, i.e. blood glucose, lactate and pyruvate - all techniques available in the laboratory - were observed.

Myocardial blood flow was estimated as previously described and the oxygen content of arterial blood was calculated. As catheters were also placed in the coronary sinus, it was possible to estimate coronary venous blood oxygen content. From these two values the arterio-venous oxygen content difference was calculated and when this (in ml. O_2/ml. blood) was multiplied by the myocardial blood flow in ml./100g./min. then the oxygen consumption was found.

Technique of Measuring Oxygen Content

Oxygen content was calculated from oxygen tensions and the haemoglobin level (details are found in the appendix). Preliminary experiments which had been carried out in the laboratory had been concerned with the reliability of the calculation type of method as compared with the Van Slyke oxygen content type of

analysis. Many readings were taken both at normal pressure and also at two atmospheres absolute and the correlation between the duplicated readings was excellent (Ledingham, 1968). Blood glucose levels were measured by the standard technique. Lactate and pyruvate levels were also estimated (see appendix) in both arterial and coronary sinus blood. From this the consumption of lactate, pyruvate and glucose was calculated as follows - consumption (mg./100g./min.) = A-V difference (mg./ml.) x blood flow in ml./100g./min.

RESULTS

11 dogs were studied in the first experiment. Table 7 gives the haemodynamic results in this series.

Table 7

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR and O_2 at 2 ATA (11 DOGS)

	Heart Rate Beats/min.	Mean Arterial Blood Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
Air ■ Mean and S.E.	160 ± 5	115 ± 5	106 ± 5
			$p < 0.001$
O_2 Mean and S.E.	155 ± 6	110 ± 7	79 ± 4.74

COMMENT

The pattern response to 100% oxygen is noted. In this case a 25% reduction in flow - a highly significant results ($p < 0.001$ by Students unpaired t-test).

Oxygen Consumption

Table 8 gives the results of myocardial blood flow, A-V oxygen content difference and the derived oxygen consumption.

Table 8

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR and O_2 at 2 ATA (11 DOGS)

	Myocardial Blood Flow ml./100g./min.	A-V O_2 Content Difference ml./100 ml.	O_2 Consumption ml./100g./min.
Air = Mean and S.E.	106 ± 5.34 p<0.001	8.8 ± 0.4	9.18 ± 0.48 p<0.001
O_2 Mean and S.E.	79 ± 4.74	8.17 ± 0.62	6.47 ± 0.55

COMMENT

Although there is a substantial fall in myocardial blood flow the A-V oxygen content difference remains virtually unaltered. The oxygen consumption is significantly reduced - the reduction being 29%.

Blood Glucose

The results regarding glucose consumpion in 9 dogs are as follows (Table 9).

Table 9

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR AND O_2 at 2 ATA (9 DOGS)

	Arterial Glucose mg./100 ml.	A-V Difference - Glucose mg./100 ml.	Glucose Consumption mg./100mg./min.
Air ■ Mean and S.E.	113 ± 8	1.9 ± 1.3	4.17 ± 1.82
O_2 Mean and S.E.	111 ± 6	3.3 ± 1.1	3.11 ± 0.77

COMMENT

Statistical analysis by both the paired and unpaired Students t-test reveals that these changes are not significant.

Blood Lactate

8 dogs were investigated with respect to blood lactate. The results are presented in Table 10.

Table 10

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW AIR AND O_2 at 2 ATA (8 DOGS)			
	Mean Arterial Lactate mg./100 ml.	A-V DIFFERENCE Lactate mg./100 ml.	Lactate Consumption mg./100mg./min.
Air = Mean and S.E.	18.3 ± 2.11	5.6 ± 0.97	5.3 ± 0.75
O_2 Mean and S.E.	18.8 ± 2.28	4.9 ± 1.88	3.0 ± 1.09

COMMENT

These figures show a reduction in lactate consumption of 43% . By Students unpaired t-test $p = 0.01$, but by the paired t-test $p < 0.001$ - highly significant.

Blood Pyruvate

Table 11 presents the results in 8 dogs.

Table 11

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR AND O_2 at 2 ATA (8 DOGS)

	Mean Arterial Pyruvate mg./100 ml.	A-V Difference Pyruvate mg./100 ml.	Pyruvate Consumption mg./100 g./min.
Air = Mean and S.E.	1.05 ± 0.15	0.28 ± 0.11	0.34 ± 0.15
O_2 Mean and S.E.	1.02 ± 0.14	0.15 ± 0.16	0.15 ± 0.04

COMMENT

The reduction in pyruvate consumption was 56% - significance level by Students unpaired t-test p = 0.05.

DISCUSSION

In the tables presented in this chapter it is to be noted that air equivalent represents a mean arterial oxygen tension of approximately 100 mm. Hg. while 100% oxygen gives an average PaO_2 of 1000 mm. Hg.

The results in summary show a reduction in oxygen consumption of the myocardium of 29%, no significant change in glucose utilisation, 43% reduction in lactate consumption, and a 56% reduction in pyruvate consumption.

Sobol, et.al. (1962) as part of an investigation into the alterations of coronary blood flow produced by inhalation of 100% oxygen investigated changes in oxygen consumption across the myocardium in four animals and a small reduction of oxygen consumption was found. From examination of their data the percentage reduction ranged from -3 to -10. No data is available about arterial oxygen tensions in this study, but as it was performed at normal atmospheric pressure and the oxygen content reached 22.9 volumes per cent, it would presumably equate to approximately 450 - 500 mm.Hg. while the inspired gas was 100% oxygen. Another feature was that determinations of consumption were made after ten minutes exposure to oxygen. It may be that longer exposure is necessary to produce changes in consumption. This factor is discussed in greater detail in Chapter 8.

Eckenhoff, et.al. (1947) had found no change in oxygen consumption with varying the inspirate from 100% to 8% oxygen. This study was at normal atmospheric pressure as was that reported by Daniell and Bagwell (1968). They reported their results regarding oxygen consumption on changing from 25% to 100%oxygen. Reductions varying from 1% to 16% were found.

In the hyperbaric field Meijne and Straub (1966) reported no change in oxygen consumption with 100% oxygen at three atmospheres absolute. Furthermore they found that glucose extraction diminished at three atmospheres absolute although lactic acid extraction remained constant.

Weglicki et.al. (1966) on the contrary found that 100% oxygen at three atmospheres absolute decreased oxygen consumption by 40 to 60% and also reported at this pressure a marked decrease in lactate and pyruvate consumption (50 to 75%). Glucose uptake was unchanged. These results are in close agreement with those presented in this chapter.

As the arterio-venous difference of oxygen content remained unchanged it may be thought that the reduction in flow is the sole determinant of the reduction in consumption. However, the mechanism of the change in consumption may be much more complex and the relationship with flow less than direct.

When taken in conjunction with the effect on lactate and pyruvate metabolism it is obvious that a direct effect on all metabolic processes may have occurred. Weglicki et.al. (1966) suggested that oxygen at high tensions presumably lowers energy requirements and possibly also lowers oxygen consumption by inhibiting lactate and pyruvate oxidation. They take this argument a step further by suggesting that hyperbaric oxygen

may inhibit pyruvate co-carboxylase or in some manner directly interfere with mitochondrial function.

The link between blood flow and oxygen consumption became more difficult to unravel when the effects of carbon dioxide were studied. This subject is introduced in the next chapter.

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Chapter 6

THE RESPONSE OF MYOCARDIAL BLOOD FLOW AND OXYGEN CONSUMPTION TO HIGH PARTIAL PRESSURES OF CARBON DIOXIDE

During the experiments on the mechanism of the reduction of blood flow to the myocardium with high partial pressures of oxygen, it was thought that variations in the partial pressure of carbon dioxide might influence this change.

It has long been thought that the pH and PCO_2 of blood flowing through a tissue are major factors in the local control of regional blood flow (Goodman and Gilman, 1955). However, scrutiny of the literature for information about the circulatory and haemodynamic effects of this gas produces conflicting evidence. With regard to the specific problem of the effect on myocardial blood flow Green and Wegria (1941) and Eckenhoff *et.al.* (1947) demonstrated no change in flow with elevated tensions of carbon dioxide (hypercapnia) while Feinberg *et.al.* (1960) showed an increase in flow.

Because of the lack of unanimity of these results, it was decided to study this problem again.

METHOD

Myocardial blood flow was measured as previously described

as was also the oxygen consumption of the myocardium. For baseline measurements, the inspired gas was a mixture of oxygen and nitrogen which was adjusted to maintain a PaO_2 of between 85 and 105 mm. Hg. Ventilation was controlled so that arterial carbon dioxide tension was kept within the range 35 to 45 mm. Hg. When it was desired to raise carbon dioxide tension, carbon dioxide gas was added to the inspired gas mixture. As a guide to the amount of carbon dioxide to be added, the inspired carbon dioxide percentage was monitored using an infra red carbon dioxide analyser (see appendix). The final tension reached was monitored by repeated measurements of arterial carbon dioxide tension. This work was performed at normal atmospheric pressure.

Experiment 1

The changes in myocardial blood flow and oxygen consumption were measured in ten dogs when arterial carbon dioxide tension was rapidly elevated from a baseline level of 35 - 45 mm. Hg. to an elevated level of 80 - 100 mm. Hg. for a short period of time.

Figure 9 demonstrates the changes in myocardial flow in 10 individual dogs when the $PaCO_2$ is rapidly elevated. In each dog there is a substantial rise in flow. The haemodynamic data from these dogs is presented in Table 12.

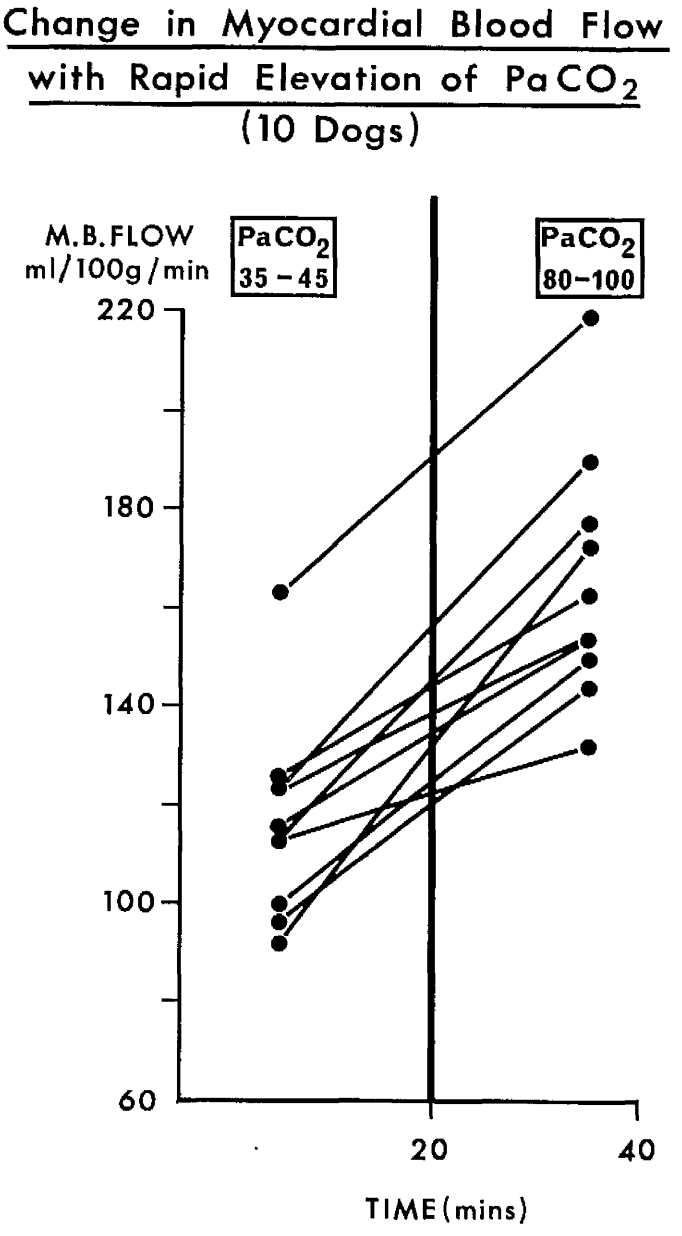

Figure 9: In each individual dog there is a substantial rise in flow.

Table 12

HAEMODYNAMIC AND pH DATA WITH RAPID ELEVATION OF $PaCO_2$ MEAN AND S.E. (10 DOGS)

$PaCO_2$ mm. Hg.	Myocardial Blood Flow ml./100g./min.	Mean Blood Pressure mm. Hg.	Heart Rate Beats/min.	pH
35 - 45	115 ± 5.9 $p < 0.001$	119 ± 5.4	163 ± 6.51	7.357 ± 0.011 $p < 0.001$
80 - 100	161 ± 8.5	118 ± 6.54	157 ± 6.12	7.058 ± 0.029

COMMENT

This table shows the haemodynamic and the pH changes in ten dogs. There is no significant change in mean blood pressure or heart rate although there is a 40% increase in the flow - a highly significant change. pH changed as expected with the elevation of carbon dioxide tension. These figures are presented in histogram form in Figure 10.

Oxygen consumption was measured in the ten dogs and the results are presented in Table 13.

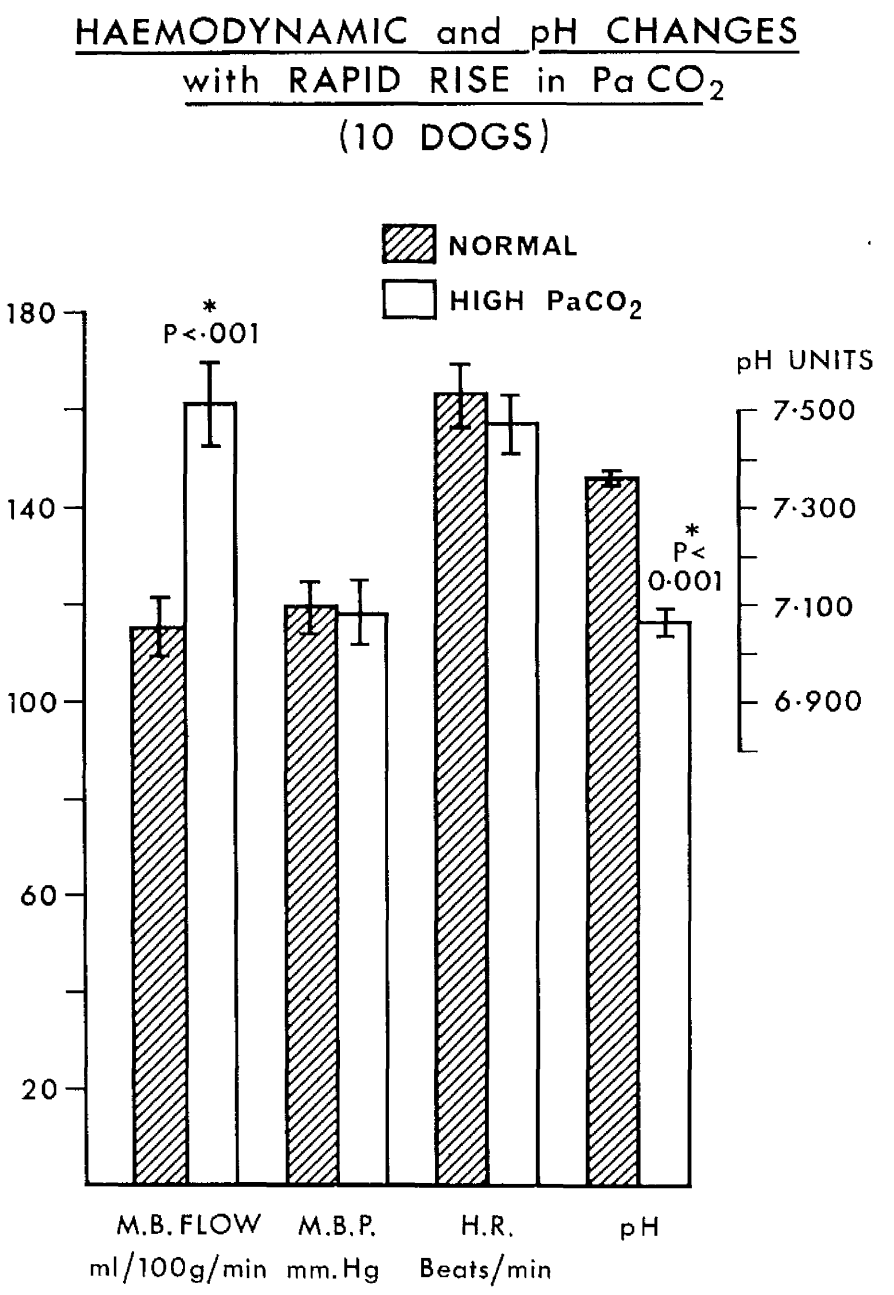

FIGURE 10: Histogram showing blood flow, blood pressure heart rate and pH changes with rapid rise in $PaCO_2$

Table 13

MYOCARDIAL BLOOD FLOW AND OXYGEN CONSUMPTION
WITH RAPID ELEVATION OF $PaCO_2$
MEAN AND S.E. (10 DOGS)

$PaCO_2$ mm. Hg.	Myocardial Blood Flow ml./100g./min.	O_2 Consumption ml./100g./min.
35 - 45	115 ± 5.9	12.91 ± 0.81
	$p < 0.001$	$p < 0.001$
80 - 100	161 ± 8.5	8.79 ± 1.08

Comment

The rise in myocardial blood flow is 40% while oxygen consumption fell by a mean factor of 32%. These changes are highly significant (Students t). The results from a typical animal in this group is shown in Figure 11.

Experiment 2

In a different series of dogs, the changes occurring in myocardial blood flow and oxygen consumption were studied when arterial carbon dioxide tension was rapidly elevated from 35 - 45

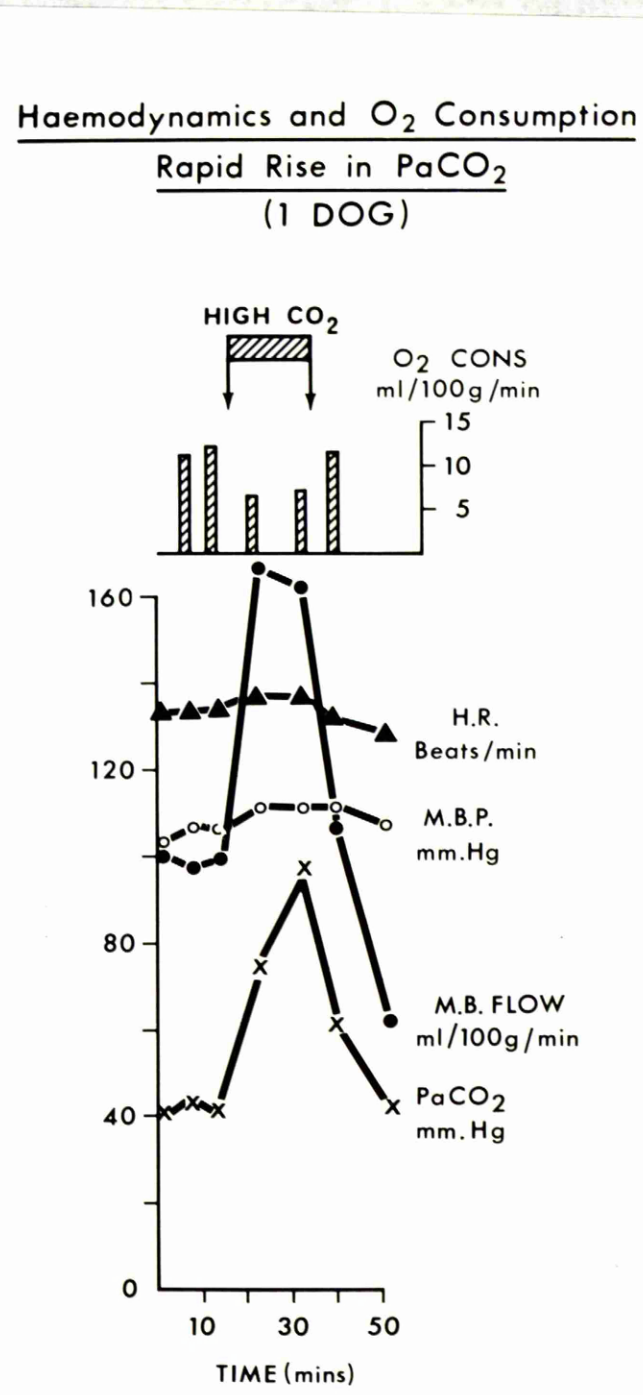

Figure 11: Haemodynamics and oxygen consumption results from 1 dog.

mm. Hg. to 80 - 100 mm. Hg. and then maintained at this level for 60 minutes. The results are presented in Figure 12. The initial column shows baseline values and succeeding columns show the means of all measurements made over consecutive ten minute periods. Mean and standard errors are shown from nine dogs for mean blood pressure and myocardial flow. The oxygen consumption data shown is from two dogs only of this series.

COMMENT

Mean blood pressure remained stable throughout. Myocardial flow rose initially with hypercapnia, but after 15 - 20 minutes began to fall again and tended to settle at a level higher than baseline. Oxygen consumption fell with hypercapnia and remained at a reduced level throughout the hypercapnic phase.

In these experiments, oxygen consumption always returned to normal after hypercapnia was corrected but myocardial flow fell below normal. After this fall below baseline level, the flow gradually rose back to baseline level over the next few minutes. Some of these points are exemplified in Figure 13. This shows the results from a single dog in the series. The overshoot of the blood flow on cessation of hypercapnia can clearly be seen. Oxygen consumption, although not shown on this diagram, quickly returned to normal.

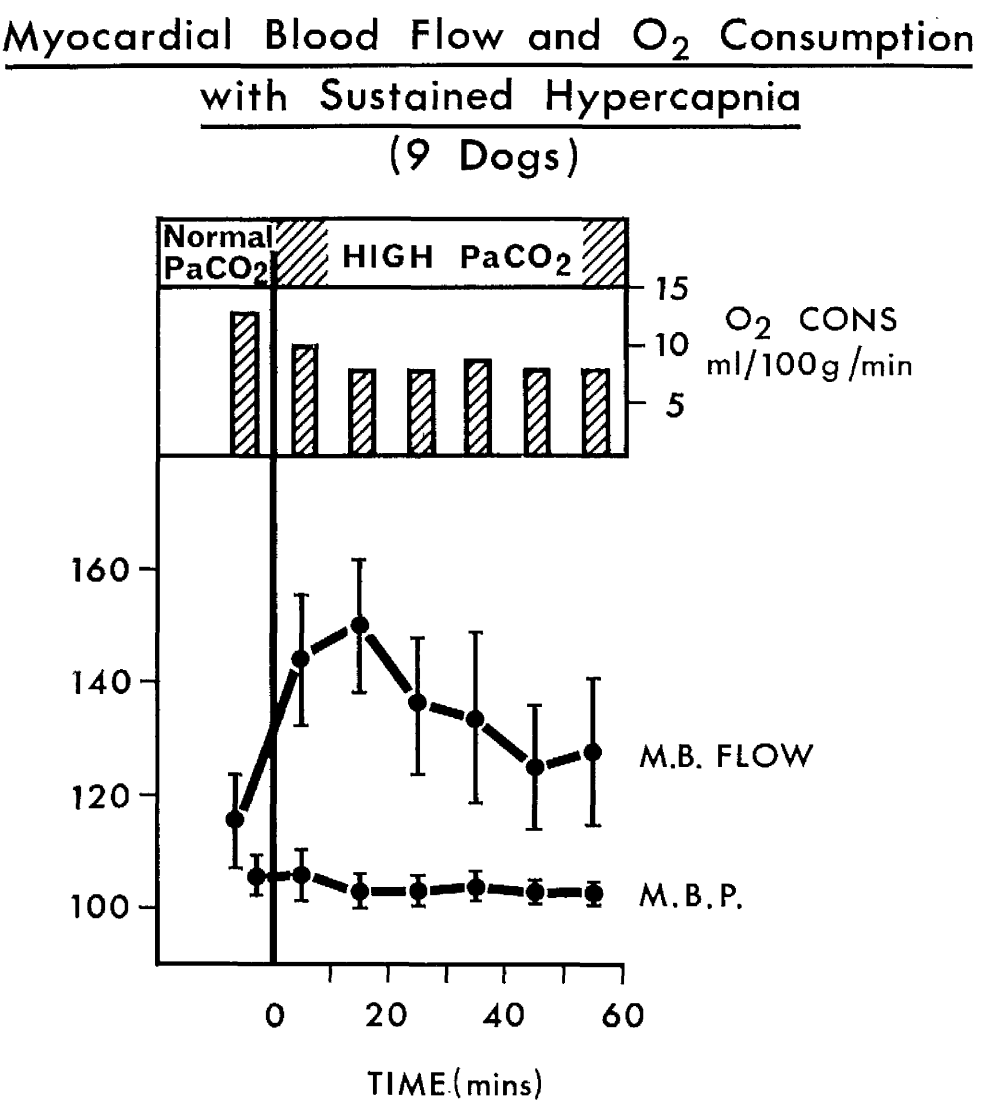

Figure 12: The oxygen consumption data in this diagram is derived from 2 dogs.

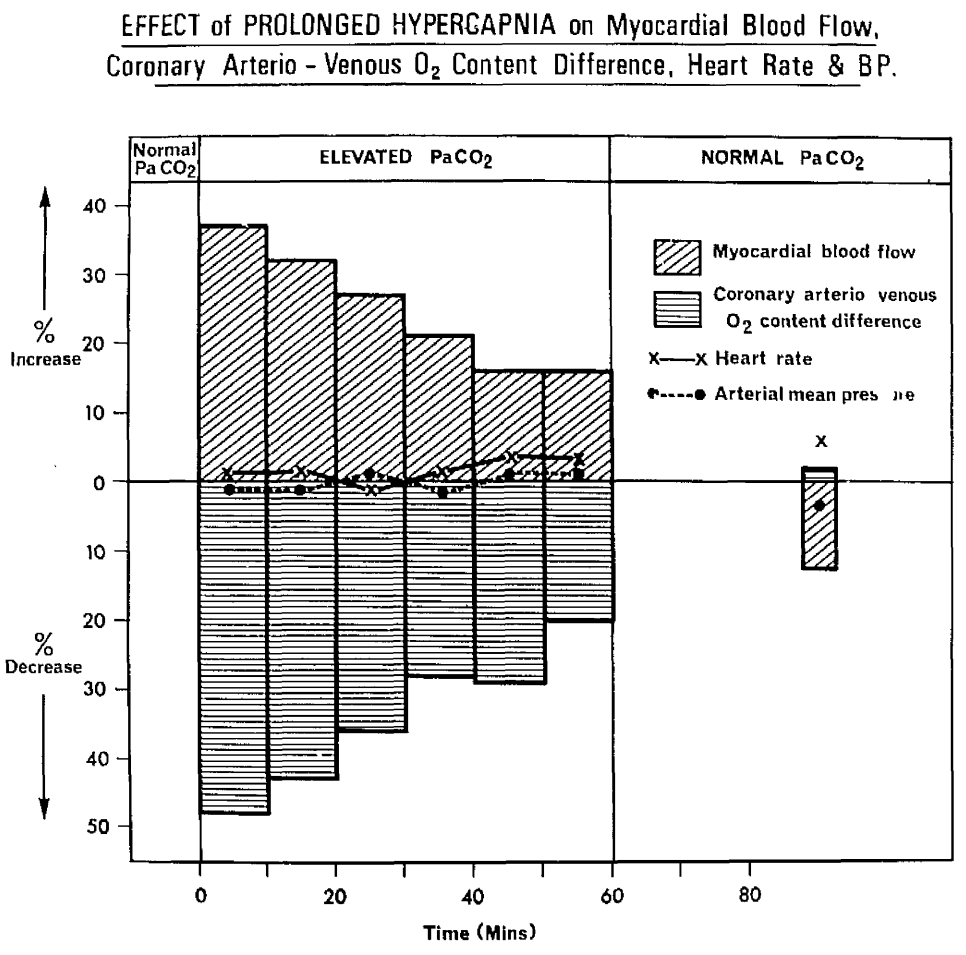

Figure 13: Effects of prolonged hypercapnia in 1 dog.

Experiment 3

To investigate the part played by changes in pH in these results, a further series of dogs was investigated in which $PaCO_2$ was held constant while a metabolic acidosis was induced by infusions of lactic or hydrochloric acid. The results obtained have been similar to those presented in Figure 14. This is from a single dog in the series. Myocardial blood flow, oxygen consumption, heart rate, mean blood pressure, arterial PCO_2 and pH are shown during and after a lactic acid infusion.

COMMENT

Lactic acidosis is associated with a rise in blood flow and a fall in oxygen consumption.

DISCUSSION

The results here presented show that raised arterial carbon dioxide tension is associated with a rise in myocardial blood flow and a fall in oxygen consumption. When hypercapnia is sustained, blood flow after the initial rise tends to fall towards baseline values whereas oxygen consumption falls and remains at its reduced level throughout hypercapnia.

These results differ from those presented by Green and

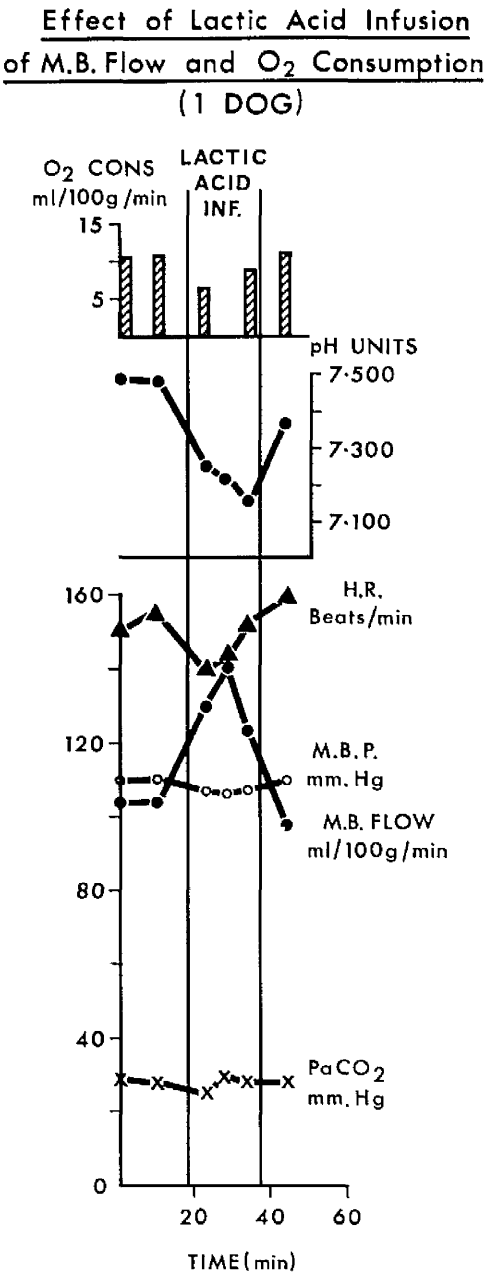

Figure 14: The effect of lactic acid infusion in 1 dog.

Wegria (1942) and Eckenhoff et.al. (1947). In the intact animal they reported absence of significant changes in mean or phasic coronary inflow after hypercapnia despite slowing of the heart and lowering of the blood pressure. Feinberg et.al. (1960) presented results which showed that coronary blood flow increased with hypercapnia and our studies are in agreement with this. However, they reported an increase in oxygen consumption with hypercapnia which is contrary to our results.

From the sustained hypercapnia results it may be inferred that hypercapnia has two different actions on the heart - one producing a transient vasodilatation in the coronary vascular bed - the other causing an alteration in myocardial metabolism. It may be that changes in pH are the important factor and some support for this is provided by the evidence from infusions of acid. This in spite of the fact that previous investigators have found acidosis to be associated with a reduction in coronary blood flow (Bing, 1965).

Whatever the mechanism underlying these effects the results presented formed a baseline for the next series of experiments with hyperbaric oxygen which are reported in the next chapter.

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Chapter 7

THE RESPONSE OF BLOOD FLOW AND METABOLISM IN THE MYOCARDIUM WHEN CARBON DIOXIDE IS ADDED TO HYPERBARIC OXYGEN

INTRODUCTION

Because of the interest aroused by the cardiovascular effects of carbon dioxide described in Chapter 6, it was decided to examine the effect of adding carbon dioxide to hyperbaric oxygen.

METHOD

The basic experimental set up was as previously described. Coronary sinus catheterisation allowed samples of coronary sinus blood to be taken for analysis of blood gases, lactate and pyruvate. Measurements of flow were recorded by the Xenon clearance technique. Heart rate, mean blood pressure were monitored as before. Readings were taken when the inspired gas mixtures were adjusted at 2 atmospheres absolute to give air equivalent, i.e. an arterial oxygen tension of between 90 and 100 mm. Hg. The inspired gas was then changed to 100% oxygen. This gave an average PaO_2 of 1000 mm. Hg. During these first two manoeuvres ventilation was adjusted to keep arterial carbon dioxide tension between 35 and 45 mm. Hg. The next procedure was to change the $PaCO_2$ abruptly by adding

carbon dioxide gas to the inspired gas mixture. In this experiment the mean level of $PaCO_2$ reached was 112 mm. Hg.

RESULTS

Table 14 presents the haemodynamic results observed during this experiment.

Table 14

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR: O_2: $O_2 + CO_2$ at 2 ATA (11 DOGS)

	Heart Rate Beats/min.	Mean Blood Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
Air = Mean and S.E.	160 ± 5	115 ± 5	106 ± 5
			$p < 0.001$
O_2 Mean and S.E.	155 ± 7	110 ± 7	79 ± 5
			$p < 0.001$
O_2 and CO_2 Mean and S.E.	161 ± 8	107 ± 7	120 ± 4

COMMENT

There is no significant change in heart rate of mean blood pressure but the fall in flow with hyperbaric oxygen is reversed by the addition of carbon dioxide, the mean increase being of the order of 50%. The statistically significant results (Students t-test) are indicated.

Figure 15 presents the changes in myocardial blood flow in the individual dogs in this experiment. In each case a substantial rise in flow occurred.

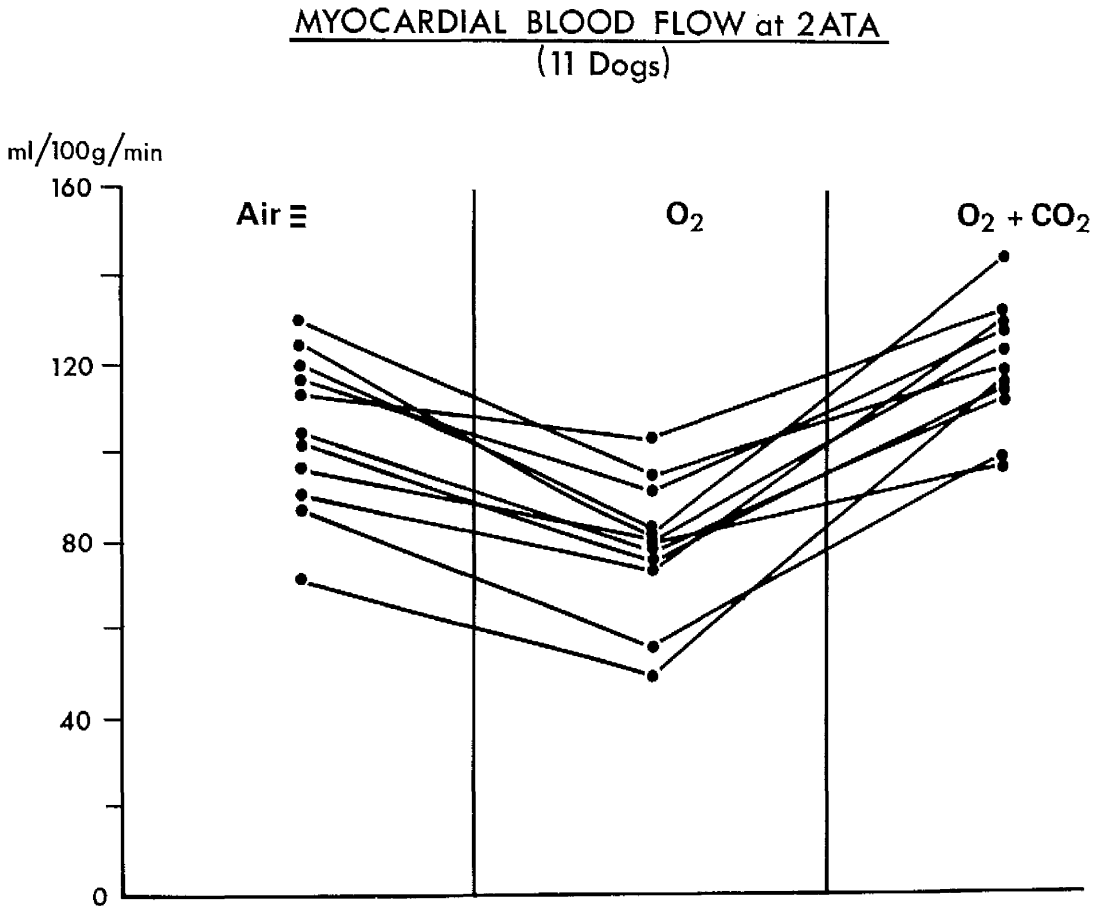
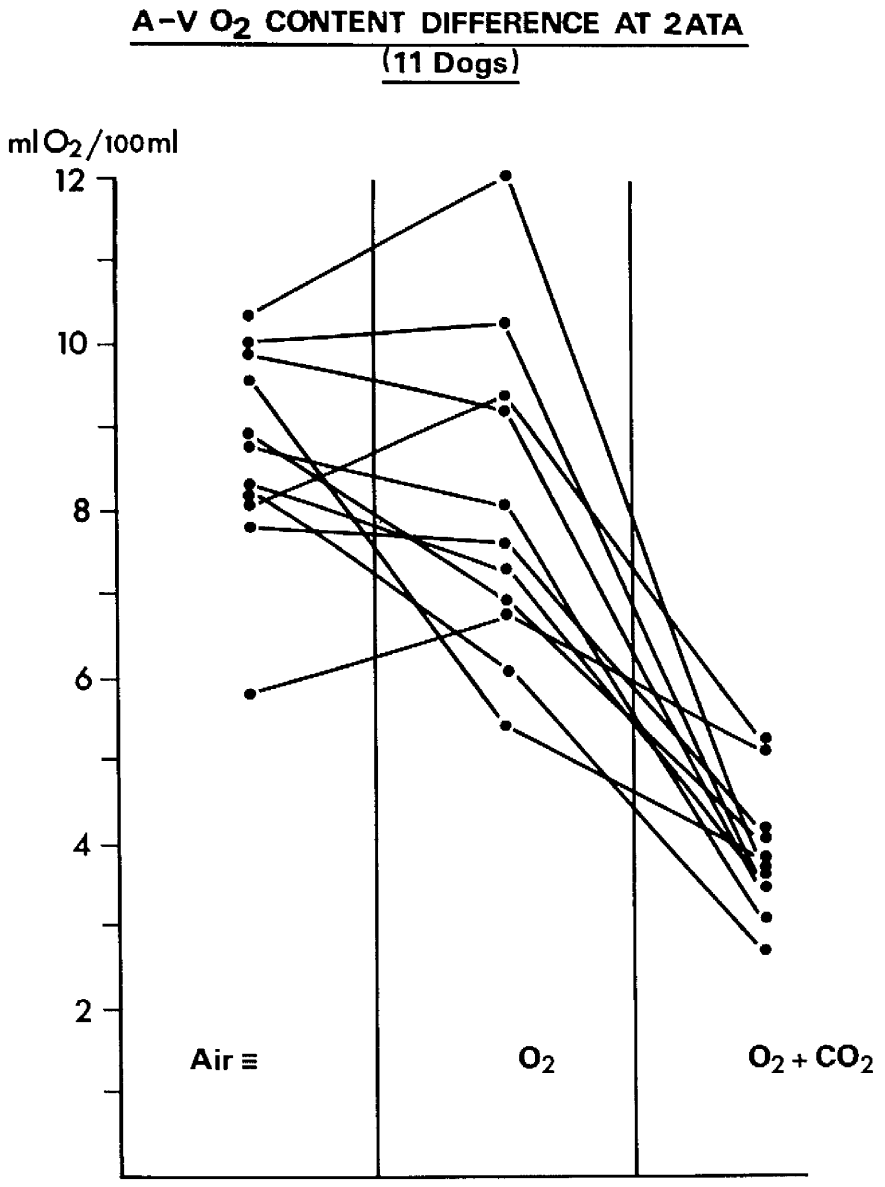


Figure 15: Myocardial blood flow in each case falls with oxygen but subsequently increases with the addition of carbon dioxide.

Figure 16 presents the changes in myocardial oxygen content difference in eleven dogs. There is no significant change in the values between air and 100% oxygen but a marked fall in all values is seen with the addition of carbon dioxide.



The values shown in the previous two figures were used to derive myocardial oxygen consumption and the results are presented in Table 15.

Table 15

MYOCARDIAL BLOOD FLOW, A-V OXYGEN CONTENT DIFFERENCE AND OXYGEN CONSUMPTION
AIR: O_2: $O_2 + CO_2$ at 2 ATA (11 DOGS)

	Myocardial Blood Flow ml./100g./min.	A-V O_2 Content ml./100 ml.	O_2 Consumption ml./100g./min.
Air = Mean and S.E.	106 ± 5	8.8 ± 0.4	9.18 ± 0.48
	$p < 0.001$		$p < 0.001$
O_2 Mean and S.E.	79 ± 5	8.17 ± 0.62	6.47 ± 0.55
	$p < 0.001$	$p < 0.001$	$p < 0.01$
O_2 and CO_2 Mean and S.E.	120 ± 4	3.87 ± 0.24	4.52 ± 0.32

COMMENT

The statistically significant changes are indicated (Students t-test). The initial fall in blood flow with 100% oxygen is reversed by the addition of carbon dioxide. A-V oxygen difference shows no change with oxygen but a substantial fall with the addition of carbon dioxide. Oxygen consumption falls with oxygen and falls by a further 30% with carbon dioxide in spite of a massive increase (50%) in flow.

LACTATE METABOLISM

The results are presented in Table 16.

Table 16

LACTATE CONSUMPTION
AIR: O_2: $O_2 + CO_2$ at 2 ATA (9 DOGS)

	Arterial Lactate mg./100 ml.	A-V Difference mg./100 ml.	Lactate Consumption mg./100 ml./min
Air * Mean and S.E.	18.3 ± 2.11	5.6 ± 0.97	5.3 ± 0.75
O_2 Mean and S.E.	18.8 ± 2.28	4.9 ± 1.88	3.0 ± 1.09
O_2 and CO_2 Mean and S.E.	16.7 ± 1.74	1.68 ± 1.42	1.94 ± 1.64

COMMENT

Lactate consumption is decreased with hyperbaric oxygen and further decreased with the addition of carbon dioxide. The changes in lactate consumption are statistically significant when examined by the paired t-test but not by the unpaired t-test.

PYRUVATE METABOLISM

The results are presented in Table 17.

Table 17

PYRUVATE CONSUMPTION
AIR: O_2: $O_2 + CO_2$ at 2 ATA (9 DOGS)

	Arterial Pyruvate mg./100 ml.	A-V Difference mg./100 ml.	Pyruvate Consumption mg./100g./min.
Air = Mean and S.E.	1.32 ± 0.11	0.34 ± 0.09	0.33 ± 0.09
O_2 Mean and S.E.	1.23 ± 0.16	0.36 ± 0.08	0.21 ± 0.07
O_2 and CO_2 Mean and S.E.	1.20 ± 0.12	0.31 ± 0.11	0.20 ± 0.10

COMMENT

Pyruvate consumption is decreased with hyperbaric oxygen although little further decrease is noted with the addition of carbon dioxide.

DISCUSSION

Carbon dioxide has been shown previously to have the ability both to increase myocardial blood flow and also to decrease oxygen consumption of the myocardium. In the situation which has been reported here, carbon dioxide was added when flow and both oxygen and substrate metabolism had already been reduced by hyperbaric

oxygen. It had the effect of further reducing oxygen and lactate consumption but still managed to increase the blood flow substantially. This gives further credence to the view that in this respect carbon dioxide has almost certainly two main types of action - a direct effect on arteriolar smooth muscle and an indirect action which occurs via metabolic pathways. If the theory of autoregulation in the coronary flow field is accepted then it may be that the indirect action which reduces oxygen consumption might favour a fall in blood flow but this is more than offset by its direct action on smooth muscle.

Oxygen consumption of the myocardium has long been considered to be a primary determinant of the level of myocardial blood flow (Berne, 1964) and these present results give rise to a paradoxical situation. There is certainly no direct relationship between consumption and flow in this situation and the most likely answer is that a pharmacological effect of carbon dioxide on the vessels has over-ridden the basic metabolic effect and given rise to these contradictory results.

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Chapter 8

CHANGES IN MYOCARDIAL FLOW AND METABOLISM WITH PROLONGED EXPOSURE TO HYPERBARIC OXYGEN

INTRODUCTION

The previous experiments which had been concerned with changes in myocardial blood flow and oxygen consumption were involved with measurements which were taken over a relatively short period. As suspicion had been aroused that hyperbaric oxygen might interfere with metabolic processes, it was decided to investigate this aspect further particularly with regard to the time of exposure to high partial pressures of oxygen. Clinical experience in various fields had led to a decision to limit, as far as possible, any one exposure to oxygen at high pressure to a maximum of three to four hours. An experiment was therefore devised in which readings would be taken first on air equivalent, then 30 minutes after changing to 100% oxygen and then three hours after changing to 100% oxygen. In this way it was felt that changes which might have escaped notice at 30 minutes may well have become obvious at 3 hours.

METHOD

METHOD

The basic experimental method was exactly as before and the only difference lay in the time of exposure to hyperbaric oxygen. Cardiac output was measured in four dogs.

RESULTS

The haemodynamic results in this different series of 11 dogs are presented in Table 18.

Table 18

HEART RATE, BLOOD PRESSURE AND MYOCARDIAL BLOOD FLOW
AIR: OXYGEN AT 30 MINUTES: OXYGEN AT 180 MINUTES
AT 2 ATA (11 DOGS)

	Heart Rate Beats/min.	Mean Blood Pressure mm. Hg.	Myocardial Blood Flow ml./100g./min.
Air = Mean and S.E.	169 ± 8	123 ± 4	118 ± 6
			p <0.005
O_2 at 30 minutes Mean and S.E.	159 ± 9	117 ± 5	87 ± 5
	p <0.05		p< 0.01
O_2 at 180 minutes Mean and S.E.	147 ± 10	99 ± 5	57 ± 4

COMMENT

Although heart rate and blood pressure show a consistent downward trend in this table, the only statistically significant change is in blood pressure between oxygen at 30 minutes and oxygen at 180 minutes. There is, however, a significant change in heart rate and mean blood pressure when comparing air equivalent readings and oxygen at 3 hours.

OXYGEN CONSUMPTION

The results relevant to oxygen consumption are presented in Table 19.

Table 19

MYOCARDIAL BLOOD FLOW, A-V O_2 CONTENT DIFFERENCE AND O_2 CONSUMPTION
AIR: O_2 AT 30 MINUTES: O_2 AT 180 MINUTES AT 2 ATA (11 DOGS)

	Myocardial Blood Flow ml./100g./min.	A-V O_2 Content ml./100 ml.	O_2 Consumption ml./100g./min.
Air = Mean and S.E.	118 ± 6	7.31 ± 0.96	8.37 ± 0.86
	$p < 0.005$		
O_2 at 30 minutes Mean and S.E.	87 ± 5	7.93 ± 0.98	6.63 ± 0.65
	$p < 0.01$		$p < 0.005$
O_2 at 180 minutes Mean and S.E.	57 ± 4	7.38 ± 0.84	4.09 ± 0.36

COMMENT

No significant change is recorded in oxygen content difference between any of the changes. Oxygen consumption steadily falls with a highly significant difference between oxygen at 30 minutes and oxygen at 180 minutes.

GLUCOSE CONSUMPTION

The results are presented in Table 20.

Table 20

GLUCOSE ARTERIAL CONCENTRATION, A-V CONTENT DIFFERENCE AND CONSUMPTION
AIR: O_2 AT 30 MINUTES: O_2 AT 180 MINUTES
AT 2 ATA (9 DOGS)

	Arterial Concentration mg./100 ml.	A-V Content Difference mg./100 ml.	Glucose Consumption mg./100g./min.
Air ■ Mean and S.E.	113 ± 8	1.9 ± 1.3	4.2 ± 1.8
O_2 at 30 minutes Mean and S.E.	111 ± 6	3.3 ± 1.2	3.11 ± 0.77
O_2 at 180 minutes Mean and S.E.	127 ± 6	2.3 ± 2.6	1.25 ± 1.42

COMMENT

The individual changes in consumption shown are not statistically significant but the change from air equivalent to oxygen at 180 minutes is highly significant.

LACTATE CONSUMPTION

The results are presented in Table 21.

Table 21

LACTATE ARTERIAL CONCENTRATION, A-V CONTENT DIFFERENCE AND CONSUMPTION
AIR: O_2 AT 30 MINUTES: O_2 AT 180 MINUTES
AT 2 ATA (8 DOGS)

	Arterial Concentration mg./100 ml.	A-V Content Difference mg. /100ml.	Lactate Consumption mg./100g./min.
Air = Mean and S.E.	14.2 ± 1.91	3. 2 ± 0.8	3.86 ± 1.12
O_2 at 30 minutes Mean and S.E.	15.2 ± 2.21	2.75 ± 1.65	2.71 ± 1.38
O_2 at 180 minutes Mean and S.E.	18.9 ± 2.15	1.94 ± 0.6	1.10 ± 0.33

COMMENT

The reduction in lactate consumption between air equivalent and oxygen at 180 minutes is 70%.

PYRUVATE CONSUMPTION

The results are presented in Table 22.

Table 22

PYRUVATE ARTERIAL CONCENTRATION, A-V CONTENT DIFFERENCE AND CONSUMPTION
AIR: O_2 AT 30 MINUTES: O_2 AT 180 MINUTES
AT 2 ATA (8 DOGS)

	Arterial Concentration mg./100 ml.	A-V Content Difference mg./100 ml.	Pyruvate Consumption mg./100g./min.
Air = Mean and S.E.	1.05 ± 0.15	0.28 ± 0.11	0.34 ± 0.15
O_2 at 30 minutes Mean and S.E.	1.02 ± 0.14	0.15 ± 0.16	0.15 ± 0.03
O_2 at 180 minutes Mean and S.E.	1.27 ± 0.18	0.14 ± 0.11	0.08 ± 0.06

COMMENT

The reduction in pyruvate consumption between air equivalent and oxygen at 180 minutes is 70%.

Cardiac Output

Cardiac output was measured in four dogs both at air equivalent and after three hours of 100% oxygen at 2 ATA. The technique used was dye dilution - in this case the dye was indocyanine green. The output in each case was reduced; the individual reductions being 23, 32, 44 and 50 per cent.

Electrocardiographic Changes

In three dogs in this series E.C.G. abnormalities appeared near the end of the three hour period of exposure to 100% oxygen. In each case the abnormality was atrio-ventricular dissociation of the interference dissociation type. Changing to air equivalent restored sinus rhythm in each case.

Changes After Cessation of Oxygen Exposure

In eight dogs in this experiment it was possible to study myocardial blood flow and oxygen consumption on resumption of air equivalent breathing after three hours of exposure to 100% oxygen. Readings were taken after 30 minutes administration of air equivalent and the results for myocardial blood flow and oxygen consumption in the individual animals are presented in the next two figures (Figures 17 and 18).

In each case a marked trend towards a return to baseline figures is noted.

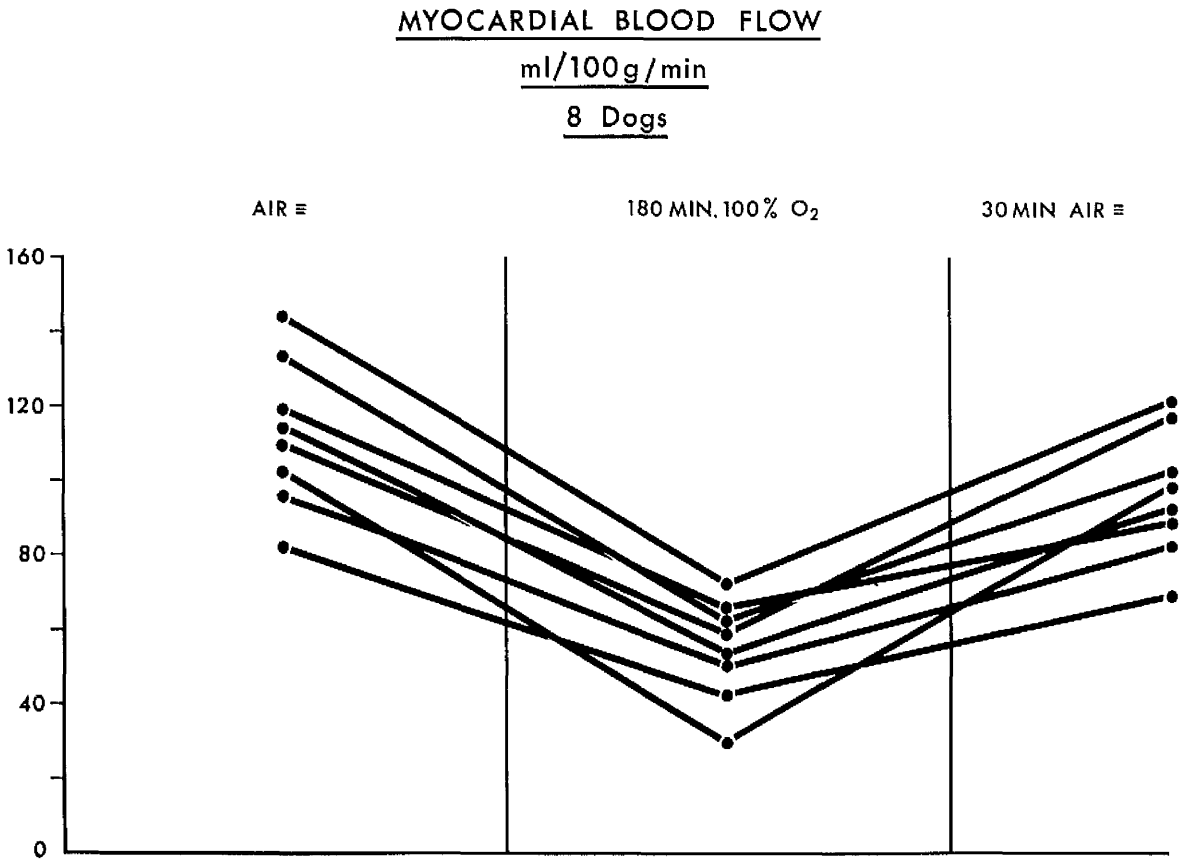

Figure 17: There is a substantial fall in flow after 180 minutes exposure to oxygen. Note the tendency to return towards normal after 30 minutes of air equivalent breathing.

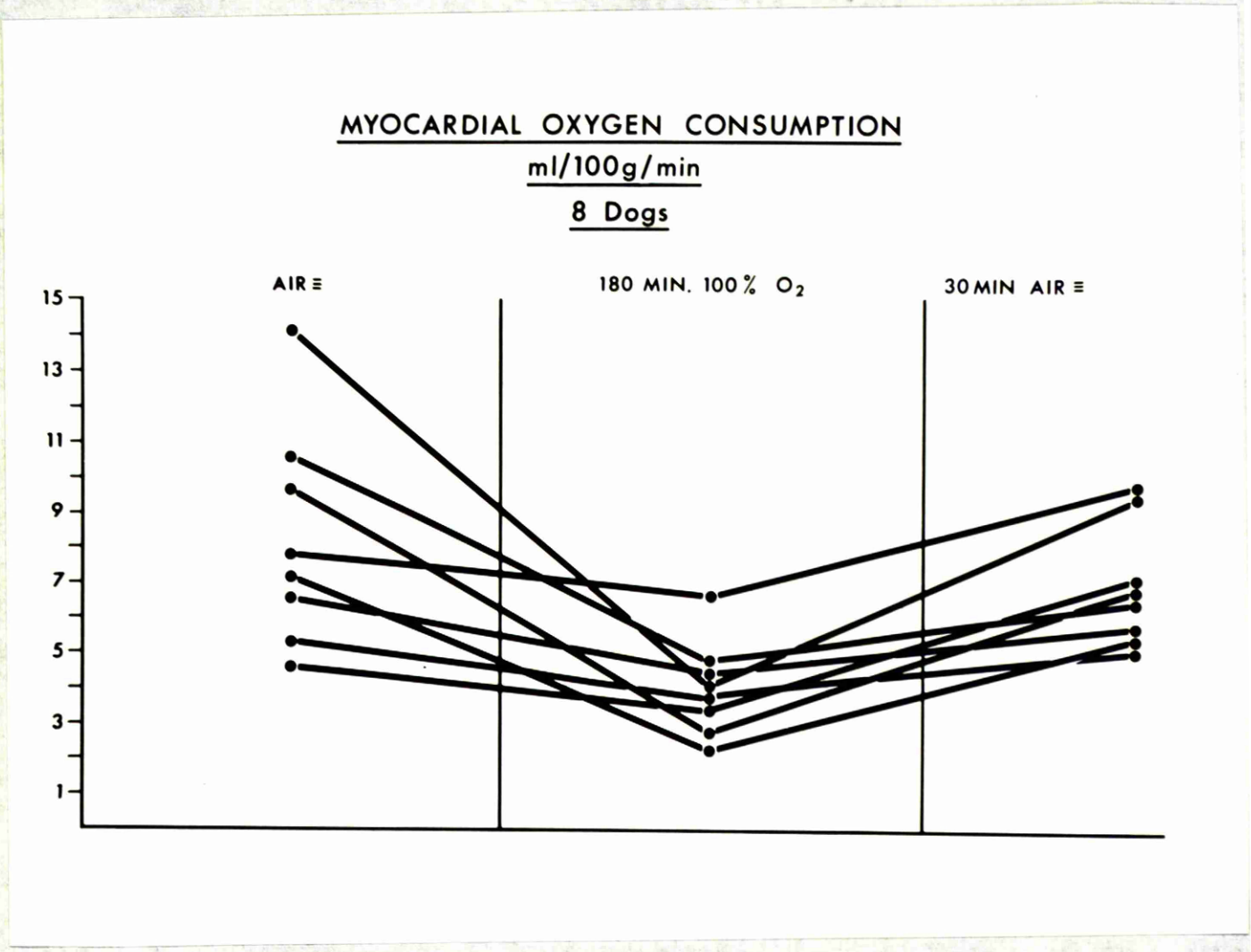

Figure 18: Myocardial oxygen consumption falls after 180 minutes exposure to oxygen, but after 30 minutes of air equivalent breathing it tends to return towards normal levels.

DISCUSSION

The results in this series of experiments have demonstrated that with prolonged exposure to high tensions of oxygen several changes occur. The myocardial blood flow falls substantially, oxygen consumption is significantly reduced and there is evidence of marked interference with carbohydrate metabolism. Other changes include a reduction in cardiac output and in some cases interference with the conduction pathways in the heart. Blood pressure and heart rate are more slightly affected. These findings raise the suspicion that there may be a toxic effect when oxygen is administered at such pressures.

The fact that oxygen may have harmful effects is no new idea. Priestley after discovering oxygen in 1775 experimented with living things and found that not only was oxygen essential to life but also remarked on its possible use as a therapeutic agent and commented on the possible danger of its use at increased concentrations. He therefore had delineated from the outset its (a) necessity, (b) therapeutic usefulness and (c) possible deleterious effects.

The first harmful effects of oxygen to be observed were described by Lavoisier (1783) who described an "incendiary" action on guinea pig lungs. This work has been confirmed many times since and in the main the pathological findings are of oedema,

hepatization of the lungs, pleural effusion, atelectasis and consolidation. There is fundamental damage to capillary and alveolar membranes - a particular feature being increased permeability which allows protein and red blood cells to traverse the walls. Vacuolization of mitochondria is said to occur. An interesting feature is that the interpretation of these changes as being due to a direct toxic action of oxygen has been challenged. Durfey (1964) thought that 100% oxygen produced profound and rapid post-mortem changes many of which had previously been ascribed to oxygen toxicity.

The changes in the lungs highlight another general effect of oxygen, i.e. that of vascular involvement. The capacity to induce vascular pathology has been amply illustrated by the tragedy of retrolental fibroplasia in human infants. In baby mice exposure to oxygen in excess, i.e. above 35 per cent, induces firstly decreased growth of blood vessels, a secondary hyperplasia on return to air breathing and finally a decrease in the number of vessels and hypoplasia which results in permanent blindness. In the adult vasoconstriction is the common reaction to exposure to high partial pressures of oxygen.

Enzyme systems are also known to be affected - including those in smooth muscle, striated muscle, heart and nerve. Rosenbaum (1963) has considered the question of enzyme inhibition by high oxygen pressure in cells. He reported that several hydrolytic

enzymes (acid phosphatase, cathepsins, beta glucuronidase) may be inhibited by short exposures to high concentrations of oxygen (e.g. 15 mins. at 7 atmospheres absolute) or longer exposures (24 to 48 hours) to lower concentrations. He thought the inhibitory effect was due to an effect on the lipid membrane forming a subcellular particle containing the bulk of activity of these acid hydrolysates. These are the lysosomes and in this case, oxygen may fix the membrane in some way. He postulates that the enzyme inhibition may be not due to destruction or modification of the enzymes but rather an interruption of the mechanism for their release.

Gerschman (1963) in reviewing the biological effects of oxygen, considers that there is no obvious demarcation line above which toxic effects begin. She considers that the fundamental toxic potentialities of oxygen reside in its molecular properties. The electron bond structure of oxygen is such that it has high oxidizing potential. It is a well qualified biological source of energy due to its high potential, abundance and availability. However, this high potential carries with it a threat to tissue integrity and life itself.

Oxygen is a sluggish oxidising agent and this fact permits its use as a potential energy source. If it were a rapid oxidising agent it would react too quickly to permit the storage of any energy. It is postulated that the reduction of oxygen takes place

via changes through free radical states - when this state is reached it reacts very rapidly. Therefore although many reactions, such as with enzymes, proceed slowly initially, they may become very rapid once enough energy is released to activate the oxygen to its free radical form. In this latter state highly destructive reactions can be started.

From this hypothesis it is presumed that the normal concentrations of oxygen (atmospheric air) is that for which we have adapted by developing adequate anti-oxidant defence systems. These may easily be overcome by supra normal pressures of oxygen. There are in fact many similarities with the initial biological effects of X - irradiation.

Haugaard (1963) has studied the effect of oxygen on enzyme systems in particular. Activity is greatly reduced in many systems and in one experiment he reported on respiration in rat heart homogenates, oxygen consumption was markedly reduced by changing from 7.4% oxygen to 100% oxygen (at atmospheric pressure). He quotes and agrees with Paul Bert who in 1878 said "Consumption of oxygen, breaking down of glucose in the blood, all chemical phenomena which can be measured easily, appear to be considerably slowed down by the action of oxygen under high tension".

Following this line of thought it would seem likely that the changes produced by prolonged exposure to hyperbaric oxygen may

indeed be manifestations of a toxic action on the cardiovascular system. Kaunitz (1942) reported evidence of myocardial damage in mice exposed to 100% oxygen and Daniell and Bagwell (1968) have suggested that the decrease in isometric systolic tension found with exposure to 100% oxygen at atmospheric pressure may be due to early oxygen toxicity. It is interesting however that Bean (1963), Donald (1965) and Balentine (1966) all writing reviews of the problems of adverse effects of oxygen have little or nothing to report on the myocardium.

It is known that different organ systems react in different ways to high levels of oxygen. In the adult human being the central nervous system is probably the most sensitive - which place the cardiovascular system occupies is at present difficult to say. The fact that in our experiments after 3 hours of hyperbaric oxygen a return to air breathing results in a rapid improvement in myocardial flow may suggest that the toxic action, if it be such, is reversible at this stage.

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SUMMARY OF RESULTS

This thesis presents the results of an investigation into the behaviour of myocardial blood flow and metabolism particularly on exposure to high partial pressures of oxygen.

It is shown that myocardial blood flow, oxygen consumption and substrate metabolism are reduced when exposed to hyperbaric oxygen. Further it is suggested that this reduction in flow is due to a direct effect of oxygen on the vessel wall.

Administration of carbon dioxide is accompanied by a depression of oxygen consumption although blood flow rises sharply.

When carbon dioxide is added to hyperbaric oxygen it is found that the reduction in flow due to oxygen is reversed although oxygen consumption continues to fall. These results, the first ever presented from this particular combination of gases, are interpreted as indicating that carbon dioxide has probably a direct action on vascular smooth muscle which outweighs any depressant metabolic effect.

Finally the effects of hyperbaric oxygen, when exposure is prolonged to three hours, are studied and the likelihood of a toxic effect of oxygen at high pressure on the myocardium is discussed.

No conclusions are to be drawn from this series involving

experimental animals regarding the human situation. This would be unwise. Work done in volunteers and also patients with myocardial infarction have however shown that oxygen does have effects - sometimes profound on the cardiovascular system.

It must be remembered that hyperbaric oxygen can be used with two aims in mind - the first to restore an abnormally low tension of oxygen to normal or to give the body enough dissolved oxygen in the plasma to maintain life until some pathological process is corrected. The second is deliberately to induce a supra normal partial pressure of oxygen in the blood, thereby hoping to benefit a particular area where tissue hypoxia exists.

Much research on the problems raised by this technique has been and continues to be performed. Although heart disease is such a common clinical problem the basic cardiovascular responses to high pressures of oxygen have not been well explored.

There is obviously a great gulf between human pathology and the experimental animal but it is felt that the results presented from this series of experiments do indicate a pattern of physiological responses which it would be unwise to ignore. Furthermore it is obvious that much more work needs to be done on human cardiovascular responses to oxygen at both normal and increased pressures so that a better understanding may be reached of the potentialities and also limitations of oxygen therapy.

ACKNOWLEDGEMENTS

The main bulk of this work (1966-68) was performed while holding an appointment as Research Physician to the Hyperbaric Oxygen Unit at the Western Infirmary, Glasgow. I am grateful both to the Medical Research Council for their financial support and facilities during this period and to Professor Kay of the Department of Surgery for encouragement and helpful criticism of this work.

I was fortunate in having Dr. Iain McA. Ledingham, Senior Lecturer in Surgery, as both supervisor and collaborator. Dr. Ledingham's wide experience both of hyperbaric oxygen and also of animal experimental work was always unhesitatingly put at my disposal.

My thanks are also due to Dr. J. P. Vance and Dr. J. Parratt, who later joined the research group and whose help and criticism have been very much appreciated.

The technical staff of the Hyperbaric Unit have been at all times patient and helpful but I should like particularly to thank Mrs. Denise Whyte who was most closely associated with this project.

I am also grateful to Mr. Gabriel Donald and his staff of the Medical Illustrations Department, Western Infirmary, for their help with the figures and diagrams.

I am indebted to Dr. F. C. Gillespie of the Regional Physics Department for invaluable help and advice with regard to the radio-active counting system.

Lastly I must acknowledge the sterling clerical help I have received from Misses M. Gibson, E. Thomson, N. Halley, Jean Kellock and Mrs. Elizabeth Nimmo.

STATEMENT OF COLLABORATION AND PUBLICATION OF WORK CONTAINED IN THESIS

The material in Chapters 2 and 3 was presented in preliminary form to the International Blood Flow Congress held in Glasgow in 1967.

> Reference: Clearance of Xenon^{133} from the myocardium as a measure of Myocardial Blood Flow with special reference to the influence on flow of increases of oxygen tension. (T. I. McBride and I. McA. Ledingham). Blood Flow through Organs and Tissues, pp. 90-100. E. & S. Livingstone, 1968.

When Dr. J. P. Vance joined the research group work was done in collaboration with him and Dr. Ledingham on the effects of carbon dioxide, i.e. part of the work presented in Chapter 6.

> Reference: Effects of changes in partial pressure of carbon dioxide on Myocardial Blood Flow. (J. P. Vance, T. I. McBride, and I. McA. Ledingham). Brit. J. Anaesthesia, 1967, 39, 688.

Dr. J. R. Parratt became associated with the group and the followinggcommunications dealt with other aspects of the material in Chapter 6.

> Reference: Effects of Raised Carbon Dioxide Tension on Blood Flow and Oxygen Consumption in the Myocardium. (J. P. Vance, T. I. McBride, J. R. Parratt and I. McA. Ledingham). Proceedings of the Scottish Society of Experimental Medicine, June, 1968.

> Reference: Effect of Hypercapnia on Myocardial Blood Flow, Oxygen Consumption and Metabolism. (I. McA. Ledingham, T. I. McBride, J. R. Parratt and J. P. Vance). Proceedings of the Physiological Society. September, 1968.

The experiments in Chapters 5 and 7 were reported as follows:

Reference: Changes in Myocardial Blood Flow and Oxygen Consumption on Exposure to Hyperbaric Oxygen. (T. I. McBride, J. P. Vance, J. R. Parratt and I. McA. Ledingham). Proceedings of the Scottish Society for Experimental Medicine, June, 1968.

Lastly the material in Chapter 8 formed the basis of the following communication given to the British Cardiac Society.

Reference: Changes in Myocardial Blood Flow and Oxygen Consumption on Exposure to Hyperbaric Oxygen. (T. I. McBride, I. McA. Ledingham and J. P. Vance). British Cardiac Society, December, 1968.

APPENDIX

Technical details of instruments and methods are herewith appended.

Blood Gas Analysis

Blood gases were measured with the Radiometer blood gas equipment (Astrup). This equipment consisted of blood gas monitor PHA 927; PO_2 electrode, E 5046/D616; PCO_2 electrode E 5036/D616; pH meter, PHM 27. The electrodes were calibrated daily with gases of known oxygen and carbon dioxide tensions. The pH meter was calibrated with two standard solutions of known pH.

Blood Gas Difference

To allow for the known discrepancy between measuring oxygen in a fluid and a gas medium the blood gas difference was calculated for each day. Blood was tonometered in a Torres rotating syringe for 30 minutes and then the oxygen tension in the gas phase was measured. The tension of oxygen in the blood phase was then measured at 15 second intervals for 2 minutes. The peak reading was used to calculate the blood gas difference and a converting factor was derived from this.

Blood Glucose

This was estimated by the standard Folin and Wu technique (J. Biol. Chem., 41, 367, 1920).

Blood Lactate

The Boehringer and Soehne method was used. Blood is first deproteinised with perchloric acid. After centrifugation buffer (0.5 M glycine) is added followed by the addition of LDH (2 mg/ml.) and 0.027 M NAD. This is incubated at 25° and then optical density is read in a Hilger spectrophotometer (Uvispek H 700) at 366 nm. After subtracting optical density of a blank the difference is multiplied by 117.5 to give mg. lactate per cent. Standard lactate concentrations were used as controls.

Blood Pyruvate

This was estimated by the Boehringer and Soehne method. Blood was deproteinised with perchloric acid. After centrifugation the supernatant is mixed with buffer (2.2 M dipotassium hydrogen phosphate). After standing in ice and subsequently being filtered, NADH (0.012 M) is added and optical density is read in a Hilger spectrophotometer at 336 nm. 0.75 LDH/ml. is then added and optical density read after 5 minutes. The difference between these optical densities is multiplied by 6.88 to give pyruvate in milligrams per cent. Standard pyruvate concentrations were used as controls.

Carbon Dioxide

The instrument used for infra red carbon dioxide analysis was the Hartmann and Braun Capnograph (URAS M).

Cardiac Output

A dye dilution technique using indocyanine green was employed. A constant rate withdrawal pump (Harvard) was used for arterial sampling. A Water's densitometer (XC 302) was used in conjunction with the Servoscribe writer and the output calculated from the graph obtained in the standard manner.

Haemoglobin

This was estimated by the cyanmethaemoglobin technique. After addition of 0.04 ml. blood to 10 ml. KCN reagent the optical density was read in the spectrophotometer (Unicam S.P. 600) at 540 mu. This was read against a standard of known haemoglobin concentration.

Oxygen Content

When the arterial oxygen tension was known and corrected for the blood gas difference of the day, the arterial oxygen saturation was read off on the Radiometer blood gas calculator (984-300). This takes into account pH, temperature and base excess. Oxygen capacity was taken as grams haemoglobin x 1.34. The percentage saturation was combined with the figure for oxygen capacity and

oxygen content was therefore derived. However allowance still had to be made for dissolved oxygen and this was calculated according to the formula; dissolved oxygen = 0.003 ml. per cent per mm.Hg. PO_2. When this figure for dissolved oxygen was added to the calculated oxygen content the total oxygen content was found.

Oxygen Percentage Analyser

Oxygen percentage was measured using a Servomex D.C.L. 101 paramagnetic oxygen analyser.

Radioactive Counting Equipment

The counting system used for measuring the gamma radiation from ^{133}Xenon was the Ekco Electronics Counting System. This consisted of H.V. high voltage supply M 5100, a pulse height analyser M 5010, rate meter M 5190 and the gamma scintillation detector M 5401. The scintillation detector used a thallium activated sodium iodide crystal. In operation the H.V. supply was normally set at 970 volts, the rate meter was set at range 300 pulses per second and the time constant normally used was 3 seconds. Pulse height analyser amplification was set at times 1 and the gain at x100. The gate width voltage was 2.0 and the threshhold voltage 4.0. The recording system used for the radioactive work was a Goerz Servoscribe type RE 511. The

sensitivity was normally set at 100 mV. and the paper speed 120 mm. per minute.

Pressure and E.C.G. Recording System

Intravascular pressures and E.C.G. were recorded on a Mingograph 81 (Elema - Schonander). The principle of this recorder is a low inertia galvanometer driving an ink jet system. This was used in conjunction with pressure transducers EMT 33 and EMT 34.

EXPERIMENTAL STUDIES ON MYOCARDIAL BLOOD FLOW AND METABOLISM WITH SPECIAL REFERENCE TO HYPERBARIC OXYGEN

by

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Summary of the thesis submitted to
The University of Glasgow, for the
degree of Doctor of Medicine

EXPERIMENTAL STUDIES ON MYOCARDIAL BLOOD FLOW AND METABOLISM WITH SPECIAL REFERENCE TO HYPERBARIC OXYGEN

SUMMARY OF THESIS

In the last few years there has been a renewal of interest in the use of hyperbaric oxygen as a therapeutic measure. Much work has been published on its use in various clinical situations and efforts have been made to use this mechanism in the treatment of different types of heart disease. It has become increasingly clear however that an understanding of the fundamental changes in myocardial blood flow and metabolism which occur on exposure to high partial pressures of oxygen was lacking. Furthermore, little well documented experimental work was available.

It was decided therefore to plan an investigation into the effects of oxygen at high pressure on myocardial blood flow and metabolism. The experimental animal was the dog and the main locus of the work was the Hyperbaric Unit at the Western Infirmary, Glasgow.

A brief review is given of the history of investigation of myocardial blood flow and a short summary of the types of methods available for such an investigation. The technique selected for measuring myocardial blood flow utilised the clearance of the radio-active gas ^{133}Xenon from the myocardium.

The theoretical basis of this method is then described and the practical details outlined. The establishment of the method in the laboratory follows.

The first experimental work concerned the effect of high partial pressures of oxygen on myocardial blood flow. These experiments were conducted at a pressure of 2 atmospheres absolute. It was shown that an abrupt change of arterial oxygen tension from 100 mm.Hg. (defined as "air equivalent") to 1000 mm.Hg. was associated with a 25% reduction in myocardial blood flow.

Although it was thought unlikely that this reduction in flow would be mediated through nervous pathways a series of experiments was devised to investigate this possibility. The change in flow was studied after (a) injections of atropine and propranolol (a beta adrenergic blocking drug), (b) injections of phenoxybenzamine (an alpha blocking drug) and (c) bretylium tosylate (an adrenergic neurone blocking drug). As the reduction in flow or changing from air equivalent to oxygen was maintained after these injections it was concluded that this reduction in flow is not reflexly caused. A direct effect of oxygen on vascular smooth muscle was thought to be responsible.

Myocardial oxygen consumption and metabolism were studied during this change from air equivalent to oxygen breathing. The metabolic parameters studied were the extraction of lactate,

pyruvate and glucose. Oxygen consumption, lactate consumption and pyruvate consumption were all substantially reduced. No change was noted in the consumption of glucose.

The next experimental work concerned the effect of high partial pressures of carbon dioxide on myocardial blood flow and oxygen consumption. This work was performed at normal atmospheric pressure. Raised arterial carbon dioxide tension was associated with a rise in myocardial blood flow and a fall in oxygen consumption.

Because of these results the effect of high arterial tensions of carbon dioxide combined with hyperbaric oxygen was studied. A further fall in oxygen and substrate metabolism occurred but a substantial increase in myocardial blood flow was noted. It is suggested that carbon dioxide may have a direct vasodilator effect on myocardial vasculature and an indirect effect which may impair cellular metabolism.

The final group of experiments concerned the changes which occurred in myocardial flow and metabolism when the change from air equivalent to oxygen was prolonged for 3 hours. The myocardial blood flow fell substantially, oxygen consumption was further reduced and evidence of marked interference with carbohydrate metabolism was found. In addition the expected fall in cardiac output was confirmed and evidence was found of interference with

atrio-ventricular conduction in the heart in three cases. These changes all tended to return to normal when administration of air equivalent was resumed. The likelihood of a toxic effect of high partial pressures of oxygen on the myocardium is discussed.

Finally a general summary of the results is presented and the clinical and laboratory implications of these studies reviewed.

This form should be completed and sent in along with the Thesis submitted by each candidate.

University of Glasgow.

DEGREE OF M.D.

TITLE OF THESIS (*In Block Letters*) EXPERIMENTAL STUDIES ON MYOCARDIAL BLOOD FLOW AND METABOLISM WITH SPECIAL REFERENCE TO HYPERBARIC OXYGE

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